COMPOUND FIXATIVES IN MICROWAVE ASSISTED TISSUE PROCESSING – A STEP TOWARDS FORMALIN FREE LABORATORY PRACTICE

DISSERTATION

SUBMITTED TO TAMILNADU DR.M.G.R. MEDICAL UNIVERSITY,

CHENNAI

in partial fulfilment of

the requirements for the degree of

M.D. (PATHOLOGY)

BRANCH - III

TIRUNELVELI MEDICAL COLLEGE HOSPITAL,

TIRUNELVELI- 627011

MAY 2018

CERTIFICATE

I hereby certify that this dissertation entitled **"COMPOUND FIXATIVES IN MICROWAVE ASSISTED TISSUE PROCESSING – A STEP TOWARDS FORMALIN FREE LABORATORY PRACTICE"** is a record of work done by **Dr.M.LAUVANYA**, in the Department of Pathology, Tirunelveli Medical College, Tirunelveli, during her postgraduate degree course period from 2015- 2018. This work has not formed the basis for previous award of any degree.

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ABBREVIATION

1.	˚C	DEGREE CELCIUS
2.	AMeX	**ACETONE-METHYL BENZOATE-XYLENE**
3.	cm	CENTIMETRE
4.	DNA	DEOXY RIBONUCLEIC ACID
5.	GHz	GIGAHERTZ
6.	MHz	MEGAHERTZ
7.	mm	MILIMETRE
8.	NBF	NEUTRAL BUFFERED FORMALIN
9.	RNA	RIBONUCLEIC ACID
10.	RT-PCR	REAL TIME POLYMERASE CHAIN REACTION
11.	UMFIX	UNIVERSAL MOLECULAR FIXATIVE
12.	VS	VERSUS
13.	ZBF	ZINC BASED FIXATIVES

DECLARATION

I solemnly declare that this dissertation titled **"COMPOUND FIXATIVES IN MICROWAVE ASSISTED TISSUE PROCESSING – A STEP TOWARDS FORMALIN FREE LABORATORY PRACTICE"** submitted by me for the degree of M.D, is the record work carried out by me during the period of 2015-2017 under the guidance of **Prof.Dr. J. SURESH DURAI, M.D**, Professor of Pathology, Department of Pathology, Tirunelveli Medical College, Tirunelveli. The dissertation is submitted to The Tamilnadu Dr. M.G.R. Medical University, Chennai, towards the partial fulfilment of requirements for the award of M.D. Degree (Branch III) Pathology examination to be held in May 2018.

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ACKNOWLEDGEMENT

This dissertation has come to your hands as a result of the combined effort of lot of people. I am thankful to all these people for helping me in bringing out this work.

I thank the DEAN **Prof.Dr. SITHY ATHIYA MUNAVARAH, M.D** for permitting me to conduct this study and to avail the resources of the hospital.

I am also thankful to my Professor and Head, Department of Pathology **Prof.DR.K.SHANTARAMAN M.D**, for his support and guidance in doing this dissertation.

I express my heartfelt gratitude to my guide **Prof.DR. J. SURESH DURAI M.D**, Professor, Department of Pathology, who has guided me through all the steps in my study right from the beginning till the end.

I am extremely thankful to the respected Professors of my department **Prof.DR.SWAMINATHAN.K M.D**, **Prof.DR.ARASI RAJESH M.D, Prof.DR.VASUKI MUTHURAMAN M.D,** and Associate Professor; **DR.V.BAGIYALAKSHMI M.D** Assistant Professors; **DR.HIDAYA FATHIMA M.D., DR.JOHNSY MERLA M.D., DR.MAHALAKSHMI M.D., DR. SINDHUJA M.D., and Dr.DINA MARY M.D.,** for their concern, valuable suggestions, and support during the study.

I also thank the lab technicians **MRS.VEERALAKSHMI, MR.SANKARANARAYANAN, MR.BALAMURUGAN, MRS.AMUTHA, MRS.MAHALAKSHMI and MR.BALACHANDER** for their support and cooperation which enormously helped me in the study. I also thank my fellow

postgraduates and my friends without whom this study would not have been possible. A special word of thanks to my family who have helped me in this journey.

I thank the almighty, for blessing me not only in this study, but in all endeavours of my life.

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PROTOCOL TITLE: COMPOUND FIXATIVES IN MICROWAVE ASSISTED TISSUE PROCESSING - A STEP TOWARDS FORMALIN FREE LABORATORY PRACTICE

PRINCIPAL INVESTIGATOR: Dr.M. LAUVANYA, MBBS.,

DESIGNATION OF PRINCIPAL INVESTIGATOR: POST GRADUATE IN PATHOLOGY
DEPARTMENT & INSTITUTION: TIRUNELVELI MEDICAL COLLEGE, TIRUNELVELI

Dear , Dr. M. Lauvanya, MBBS., The Tirunelveli Medical College Institutional Ethics Committee (TIREC) reviewed and discussed your application during the IEC meeting held on 05.08.2016.

THE FOLLOWING DOCUMENTS WERE REVIEWED AND APPROVED

1.	TIREC Application Form	☐
2.	Study Protocol	☐
3.	Department Research Committee Approval	☐
4.	Patient Information Document and Consent Form in English and Vernacular Language	☐
5.	Investigator's Brochure	☐
6.	Proposed Methods for Patient Accrual Proposed	☐
7.	Curriculum Vitae of the Principal Investigator	☐
8.	Insurance /Compensation Policy	☐
9.	Investigator's Agreement with Sponsor	☐
10.	Investigator's Undertaking	☐
11.	DCGI/DGFT approval	☐
12.	Clinical Trial Agreement (CTA)	☐
13.	Memorandum of Understanding (MOU)/Material Transfer Agreement (MTA)	☐
14.	Clinical Trials Registry-India (CTRI) Registration	☐

THE PROTOCOL IS APPROVED IN ITS PRESENTED FORM ON THE FOLLOWING CONDITIONS

1. The approval is valid for a period of 2 year/s or duration of project whichever is later
2. The date of commencement of study should be informed
3. A written request should be submitted 2weeks before for renewal / extension of the validity
4. An annual status report should be submitted.
5. The TIREC will monitor the study
6. At the time of PI's retirement/leaving the institute, the study responsibility should be transferred to a person cleared by HOD
7. The PI should report to TIREC within 7 days of the occurrence of the SAE. If the SAE is Death, the Bioethics Cell should receive the SAE reporting form within 24 hours of the occurrence.
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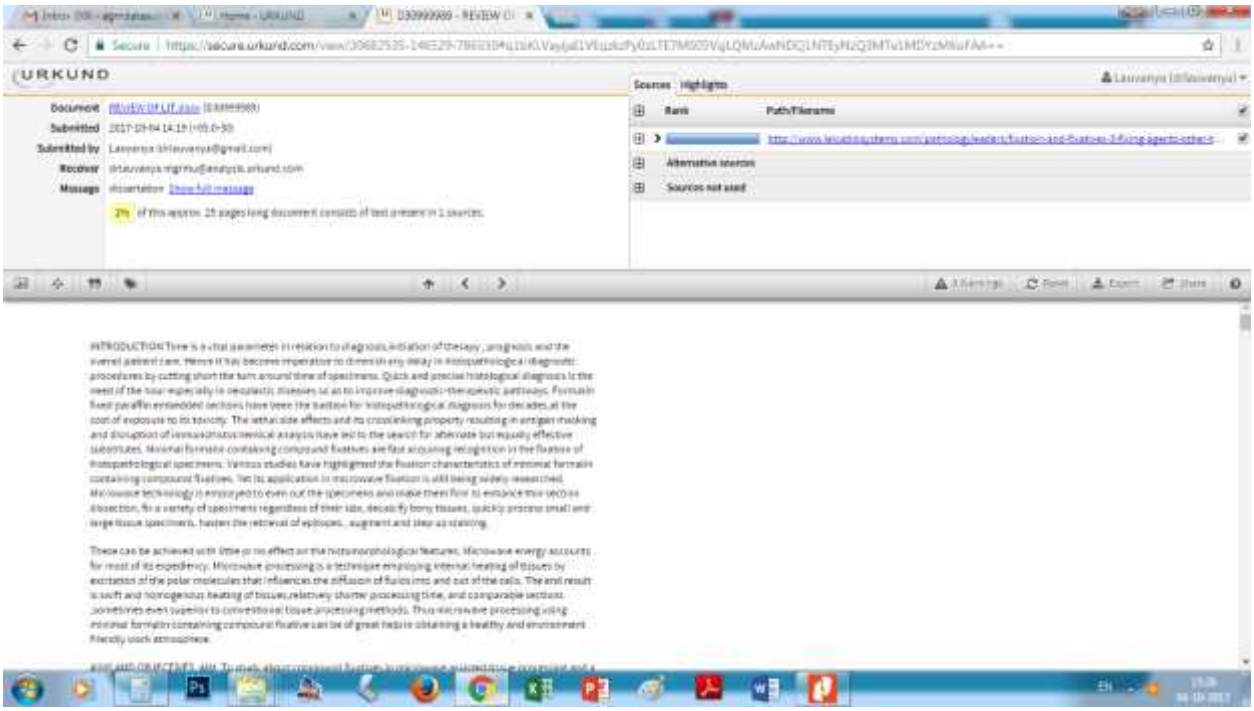
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INTRODUCTION

Time is a vital parameter in relation to diagnosis, initiation of therapy, prognosis and the overall patient care. Hence it has become imperative to diminish any delay in histopathological diagnostic procedures by cutting short the turnaround time of specimens. Quick and precise histological diagnosis is the need of the hour especially in neoplastic diseases so as to improve diagnostic-therapeutic pathways.

Formalin fixed paraffin embedded sections have been the bastion for histopathological diagnosis for decades,at the cost of exposure to its toxicity. The lethal side effects and its crosslinking property resulting in antigen masking and disruption of immunohistochemical analysis have led to the search for alternate but equally effective substitutes. Minimal formalin containing compound fixatives are fast acquiring recognition in the fixation of histopathological specimens.

Various studies have highlighted the fixation characteristics of minimal formalin containing compound fixatives. Yet its application in microwave fixation is still being widely researched.

Microwave technology is employed to even out the specimens and make them firm to enhance thin section dissection, fix a variety of specimens regardless of their size, decalcify bony tissues, quickly process small and large tissue specimens, hasten the retrieval of epitopes , augment

and step up staining. These can be achieved with little or no effect on the histomorphological features.

Microwave energy accounts for most of its expediency. Microwave processing is a technique employing internal heating of tissues by excitation of the polar molecules that influences the diffusion of fluids into and out of the cells. The end result is swift and homogenous heating of tissues, relatively shorter processing time, and comparable sections, sometimes even superior to conventional tissue processing methods.

Thus microwave processing using minimal formalin containing compound fixative can be of great help in obtaining a healthy and environment friendly work atmosphere.

AIMS AND OBJECTIVES

AIM:

To study about compound fixatives in microwave assisted tissue processing and a step towards formalin free laboratory practice.

OBJECTIVES:

- To evaluate the fixation characteristics of a new (minimal formalin containing alcoholic) compound fixative using microwave.
- To diminish formalin exposure in those handling the histopathological specimens.
- To reduce processing time using microwave.

REVIEW OF LITERATURE

Conventional tissue fixation and processing are time proven methods which are considered the norms against which all newer technologies need to be equated. Turnaround time has been a significant concern for many years and has turned out to be exceedingly important.

Microwaves, a form of electromagnetic wave, when utilized in histotechnology, constantly yields histologic material of similar or superior quality to that provided by traditional processing methods, making it more sought after in the recent times.

FIXATION:

The living cell is in a fluid or a semifluid state.[1]The pathologist tries to stabilize the apparent microanatomy of tissue by the process of fixation.Fixation is the foremost step in tissue preservation for pathological diagnosis and its purpose is to maintain tissues enduringly in as life-like a state as possible.[2]Fixation of tissues is fundamental for successful dissection, processing and microscopic examination of histopathology specimens.

PRINCIPLE OF FIXATION :

Fixation leads to denaturation and coagulation of proteins in tissues. The fixatives form cross links between proteins, thereby producing a gel, keeping everything in their in vivo relation to each other.

AIM OF FIXATION:

1. To preserve the tissue as if like life as possible.
2. **Hardening :** The hardening action of fixatives permits easy treatment of soft tissue like brain, intestines etc.
3. **Solidification:** Changes the semifluid consistency of cells to an irreversible semisolid consistency.
4. **Optimum Optical differentiation**[2] :

   Fixation alters the refractive indices of the different components of cells and tissues so that components that are not stained are more easily visualized than when unfixed.
5. **Effects on staining**:

   Provides a mordanting effect, thus facilitating successive staining of tissues.

   Improves cell avidity for special stains.
6. To avert postmortem changes like autolysis and putrefaction.
7. Preservation of chemical compounds and microanatomic constituents so that auxiliary histochemistry is feasible.

CHARACTERISTICS OF A GOOD FIXATIVE:

- Have quick penetration.
- Be cheap, stable and safe
- Segregation of proteins, RNA and DNA with not much noteworthy biochemical alterations ought to be possible.[3]
- Create only a minimal physical and chemical alteration of the tissues.
- Must cater to assorted variety of specimens.
- Must sustain histochemical as well as immunohistochemical studies and other specialized procedures.
- Be attuned with modern automated tissue processors.
- Subsist for long term, easily disposable or recyclable and also provide brilliant microtomy of embedded blocks.

CONDITIONS INFLUENCING FIXATION:

1. VOLUME:

The volume of fixative is important. It should be at least 10 times the volume of the specimen.[4] Changing the fixative at intervals helps to evade exhaustion of the fixative. Agitation of the specimen in the fixative will also enhance fixation.

2. CONCENTRATION OF FIXATIVE:

The appropriate concentration of fixatives is influenced by the effectiveness and solubility of the fixative. Formalin is best at 10%; Glutaraldehyde is generally made up at 0.25% to 4%. Too high a concentration may adversely affect the tissues and produce artifact similar to excessive heat. Ethanol below 70% does not eliminate free water from tissues efficiently.

3. pH

Fixation is best carried out close to neutral pH, in the range of 6-8. Hypoxia of tissues lowers the pH, so there must be buffering capacity in the fixative to prevent excessive acidity. Common buffers include Phosphate, Bicarbonate, Cacodylate, Tris, and Acetate.[3]

4. TEMPERATURE:

Increasing the temperature will augment the speed of fixation. Hot formalin fixes tissues faster. The diffusion of molecules becomes greater with rising temperature due to their more brisk movement and vibration. Hence microwaves are used now to speed up fixation and tissue processing.

5. PENETRATION:

Penetration of tissues depends upon the diffusability of each individual fixative. Formalin and Alcohol penetrate the best, and Glutaraldehyde the worst. Mercurials and others are anywhere in between. Sectioning the tissues thinly (2 to 3 mm) reduces this problem. Penetration into a thin section will occur more rapidly than for a thick section. To allow proper penetration of fixatives from all directions, the bottom of the container should be wadded by fixative-soaked cotton or cloth and the specimens placed over that. Bloody gross specimens ought to be washed prior to putting into fixative.

6. OSMOLALITY:

Hypertonic and hypotonic solutions lead to cell shrinkage and swelling respectively. The better morphological details are obtained with solutions that are slightly hypertonic (400- 450 mOsm). The ionic compositions of solutions must be as isotonic as possible to the tissues.

7. TIME INTERVAL FROM REMOVAL OF TISSUES TO FIXATION:

Ideally, tissues should be fixed immediately and completely from the living state. Prefixation time refers to the time lapse from the surgical excision of the specimen to the fixative.[1]Major biochemical alterations occur in tissues within 10 minutes subsequent to anoxia.[5]

Hence, the prefixation time should be kept slightest to minimize RNA and protein degradation. The faster the tissue is obtained and fixed, the better. If tissue is left outside, artifacts will be instigated by drying. Hence they should be kept moist with saline. The longer the wait, larger is the loss of cellular organelles. More nuclear shrinkage and artefactual clumping also transpires.

FIXATION METHODS:

PHYSICAL :

- Heat fixation
- Freeze drying and freeze substitution.
- Microwave fixation

CHEMICAL :

- Cross linking fixatives
- Coagulant fixatives
- Compound fixatives

PHYSICAL METHODS:

HEAT FIXATION:

It is the simplest form of fixation. Boiling of tissues in normal saline can produce adequate morphology of the tissue. In recent times, heat is mainly used to speed up other forms of fixation and used in the tissue processing steps.

FREEZE DRYING AND FREEZE SUBSTITUTION:

In freeze drying, thin sections are prepared and engrossed in liquid nitrogen. The water molecules are then extracted in a vacuum chamber at -40˚c.[4]It is valuable for analysing small and soluble particles.

In freeze substitution, at – 40˚c, tissues are immersed in fixatives that lead to slow elimination of water molecules by dissolution of ice crystals. Continuing raise in temperature to 4˚ c concludes the fixation process.[4]

MICROWAVE FIXATION:

Microwave is an electromagnetic non-ionizing wave with a frequency (300 MHz to 300 GHz)[6] and wavelength that can be found intermediate between a radio wave and visible light in the electromagnetic spectrum.[7]Microwave heating accelerates fixation. It can lessen fixation time from 12 hours to 20 minutes.

CHEMICAL FIXATION:

Organic and inorganic compounds are used for good morphological preservation.

CROSS LINKING FIXATIVES:

Formaldehyde

Glutaraldehyde

Other aldehydes: Chloral hydrate and Glyoxal

Salts of metals such as Mercuric and zinc chloride.

Metallic compounds such as osmium tetroxide.

FORMALDEHYDE:

Formaldehyde is a naturally occurring gaseous compound. The liquid form branded as formalin is made up of 37-40% of formaldehyde and 60-63% of water.[8]

The fixative action of formaldehyde is most likely to be wholly due to its interactions with proteins. The aldehyde group merges with nitrogen and other atoms of proteins producing a cross-link -CH2- known as a methylene bridge.[8]

BENEFITS OF FORMALIN FIXATION:

- Cheap.
- Easy preparation.

- Reasonably stable.
- Frozen sections can be produced from formalin fixed material.
- Fat staining can be effortlessly carried out on tissues fixed in formalin.
- Penetration is good and does not produce excessive hardening.
- Re-establishment of natural tissue colors through formalin fixation.

DRAWBACKS:

- Irritant.
- Leads to Allergic contact dermatitis.
- Unbuffered formalin causes dark brown artifact pigment.
- Inappropriate for electron microscopy
- Slow fixation (16–24 hours) prevents intra operative decision making.[31]
- Sluggish quenching of enzymatic activity results in RNA degradation.[31]

ADVERSE EFFECTS:

International Agency for Research on Cancer has reported a powerful alliance between formaldehyde contact and human nasopharyngeal carcinoma.[57] A connection between formaldehyde contact and myeloid leukemia was recognized in 2009.[52]

Formaldehyde was initially quoted in the Second Annual Report on Carcinogens in 1981 as “reasonably anticipated to be a human carcinogen”

and was then listed as “known to be a human carcinogen” in the 12th edition National Toxicology program(2011).[10]

The frequent noxious effects of formalin are Headache, Throat discomfort, and Breathing difficulties.

COAGULANT FIXATIVES:

Frequently used coagulant fixatives are Alcohols like Ethanol and Methanol.

MECHANISM OF ACTION:

Coagulant fixatives operate by denaturing and precipitating proteins. This is done by elimination of free water molecules and interruption of the hydrogen bonds.

Free water molecules regularly surround hydrophobic areas of proteins and by repulsion, the water molecules drive hydrophobic areas closer and thereby stabilize hydrophobic bonding. When alcohol removes water, this hydrophobic bonding weakens. Water molecules also take part in hydrogen bonding in hydrophilic areas of proteins. So water removal in alcoholic fixation weakens this hydrogen bonding. All these changes upset the tertiary structure of proteins.

Coagulant fixation begins at a concentration of 50–60% for Ethanol but requires a concentration of 80% or more for Methanol.[3]

EFFECT ON TISSUES:

Various studies have shown that noncross linking alcoholic fixatives constantly provided better-quality results for nucleic acid fixation than aldehydes[26,27]Ethanol and methanol conserve nucleic acids better for the reason that they lead to petite chemical changes. Physical and chemical measurements have revealed that DNA is largely warped in Ethanol (65% v/v) and Methanol.[1]When the denatured DNA is rehydrated there is reversion to the original structure.[27]

Hundred percent ethanol and methanol are acknowledged to be supreme fixatives for preserving both high-molecular weight DNA and RNA. The low molecular weight and swift tissue penetration of alcohol is thought-out to be accountable for the uniform tissue fixation and insignificant loss of tissue components.[26]

OTHER COAGULANT FIXATIVES:

PICRIC ACID AND TRICHLOROACETIC ACID

Both Picric acid and Trichloroacetic acid may introduce a lipophilic anion into a hydrophilic region and thereby upset the tertiary structure of proteins.

COMPOUND FIXATIVES:

Amalgamation of coagulant fixatives with non-coagulant cross linking fixatives is known as compound fixatives.

As no fixative is efficient in conserving all the tissue constituents, combination of fixatives are used in compound fixatives. This helps in rectifying the inadequacies of one fixative by other. The addition of formaldehyde to dehydrating agent like ethanol results in lesser amount of shrinkage and hardening compared to pure dehydrating agents and it is proficient in preserving molecules such as glycogen. Fixation of tissues in alcoholic formalin might be of assistance to discriminate lymph nodes entrenched in fat.

- **AMeXfixative**:

  Content:

  Acetone

  Methyl benzoate

  Xylene

  DNA and RNA removal was good and similar to fresh frozen specimens.[64]

- **BOUINS FLUID :**

  Contents:

  Picric acid

Glacial acetic acid

40% Formaldehyde

Advantages:

Homogeneous and speedy permeation with no shrinkage.

Most dependable for testicular biopsies.

- **CARNOY'S FIXATIVE:**

Content:

Ethanol

Chloroform

Glacial acetic acid

Advantage:

Conservation of nucleic acid in specimens is good.

Disadvantage:

Shrinkage and hardening of tissues

- **CLARKE'S SOLUTION:**

Content:

Ethanol

Glacial acetic acid

- **FINEFIX:**

Content:

Ethanol

Polyvinyl alcohol

Glycerol

Monomeric carbohydrates.

Advantages:

Also called as Soft-Fix, a formalin-free fixative prepared in 70% ethanol. Produces shorter duration of fixation. Therefore histological artifacts produced by alcohol based fixatives are absent.DNA and RNA integrity is conserved well. Used for microwave and conventional fixation.

- **MODIFIED METHACARN:**

Contents:

Methanol

Chloroform

Glacial acetic acid.

RNA Conservation was good with this fixative.[38]

- **OMNIFIX** :

Content:

Ethanol

Ethylene glycol

Acetic acid

Sodium chloride

Zinc chloride.

Fixation is brought about by water removal, precipitation of nucleic acid and stabilization of protein configuration.

- **PAGA:**

Content:

Polyethylene glycol

Acetic acid

Ethanol

Glycerol.

Nuclear characteristics are better conserved.

- **RCL 2:**

Content:

Ethanol

Acetic acid

Complex carbohydrate.

Efficiency and conservation of morphological features are similar to formalin fixation.

- **Universal molecular fixative**:

Content:

Methanol

Polyethylene glycol.

Generally clubbed with microwave assisted tissue processing. It is immensely useful in RT-PCR for small biopsies.

- **ZENKER'S FLUID:**

Content:

Mercuric chloride

Potassium-di-chromate

Sodium sulphate

Glacial acetic acid.

Advantages:

Uniform permeation and swift fixation

Disadvantages:

The tissue must be washed in running water after fixation to remove excess dichromate. Mercury pigment must be removed with Lugol's iodine.

- **ZINC BASED FIXATIVES:**

Content:

Zinc acetate

Zinc chloride

Calcium chloride in Tris buffer.

Advantage:

DNA and protein evaluation is better in variety of tissues.

Efficient, cost effective, and relatively non-toxic fixative.

Disadvantage:

Shrinkage of tissue

Histology might be modified

Suresh Durai et al have used a minimal formalin containing compound fixative known as the new compound fixative.[9]

Composition of new compound fixative[9]:

Ethanol -20ml

Formalin-7ml

Glycerin-5 ml

Methylene blue-0.05g

Buffer - 4g of Sodium dihydrogen phosphate monohydrate

6g of anhydrous disodium hydrogen phosphate

0.7% hypotonic saline was used to make up the volume to100 ml. The PH adjusted between 7. 2 - 7.4.

Cell shrinkage may be caused by Ethanol. This can be avoided by reconstituting in hypotonic saline. Evaporation was curtailed by addition of Glycerin. The fixative was light blue in color due to usage of Methylene blue. This helped to scrutinize the color of fixatives and to shun the inclination to smell the components.

100 unfixed tissue slices were fixed in new compound fixative as well as in 10% NBF using microwave. Stained slides were scrutinized using light microscope.

Architecture, nuclear features, cytoplasmic particulars, fixation artifacts and staining characteristics, were observed. Scoring was given for the nuclear, cytoplasmic and architectural features from 0- 9.

Nuclear parameters such as preservation of nucleus and nucleolus, size of the nucleus, nuclear membrane regularity and mitotic figures were considered. Score 3 was assigned to specimens fixed in new compound fixative with nuclear characteristics comparable to specimens fixed in 10% NBF. Score 2 was assigned to sections having 1 to 2 less distinct nuclear features. Score 1 was assigned to sections having more than 2 less distinct nuclear parameters. Score 0 was assigned to sections having poor conservation of parameters and was not suitable for diagnosis.

Cytoplasmic characteristics like color, volume, nuclear cytoplasmic contrast and erythrocyte integrity were scrutinised. Score 3 was assigned to specimens fixed in new compound fixative with cytoplasmic characteristics analogous to specimens fixed in 10% NBF. Score 2 was assigned to slices with cytoplasmic shrinkage and less evident cytoplasmic granules. Score 1 was assigned to slices with more than 2 less definite cytoplasmic features. Score 0 was assigned to sections with poor maintenance of particulars.

Architectural variables such as shrinkage artifacts, distortion, cracking and formalin pigments were evaluated. Score 3 was assigned to specimens fixed in fixatives with architectural features parallel to tissues fixed in 10% NBF. Score 2 was assigned to sections with 1 to 2 less distinct architectural variables. Score 1 was assigned to sections with more than 2 less distinct architectural variables. Score 0 was assigned to sections with poor preservation of details which was unsuitable for diagnosis.

MICROWAVE FIXATION:

Electromagnetic waves are classified based on their frequencies:

- Radio waves
- Television signals
- Radar beams
- Infrared waves
- Visible and Ultraviolet light
- X-rays and Gamma rays.

Electromagnetic waves having a frequency between 300 MHz and 300 GHz are known as Microwaves. Both these frequencies equate to wavelengths of 1 m and 1 mm, correspondingly.

HISTORY OF MICROWAVES IN HISTO PROCESSING:

The phrase microwave has been found in text in the first issue of Alta Frequenza.[11]. The Magnetron was designed at the GE Research Laboratory in 1916. The microwave oven was discovered in 1945 and US patent award was obtained in 1950. Microwave irradiation was first engaged as a fixation method in the laboratory in 1970.[12] Login was the earliest to get satisfactory outcome in microwave fixation of surgical and autopsy slices.[12]Microwavetechnique was put to use in tissue processing in 1985 by Kok and Boon from Netherlands and Anthony Leong from Australia.[12] The first microwave histoprocessor was released to the world by Milestone technology in 1990s.[13]

Microwave technology emerged from the expansion of radar (Radio Detection and Ranging). Microwave pulses can be used for distance and time measurement, as they are very short. The basic variety of radar calculates the time for an echo to come back from a specific direction. Microwaves pierce fog and clouds, pass through in straight lines, and offer distinctive shadows and reflections.

Microwaves are able to pass all the way through substances with tiny or no effect, or they are reflected or absorbed. Substances such as plastics and glass, are considered “microwave transparent” since they remain unaltered on contact to microwaves. Some like metal, will reflect microwaves. As

soon as substances take up microwave energy, they become animated and create internal heat. Tissues absorb the Microwave energy and transform it into kinetic and chemical energy.

Based on this principle, substances are classified as:[16]

1. Microwave transparent, e.g.: Plastic, Glass, Paraffin pellets

2. Microwave reflectant, e.g.: Metals

3. Microwave absorbent e.g.: Tissues, Proteins

PRINCIPLE OF MICROWAVE:

Microwaves speedens fixation and processing of tissues.

The effect of microwave energy on the tissues and the mechanism by which they act is not entirely elucidated.

- Microwaves produce internal heating by stirring molecules to spin.
- Rotation generates heat energy.[14]
- Heat intensifies the rate of diffusion of fluids into and out of tissue blocks or sections.[13]Heating was used in histoprocessing to attain enhanced diffusion and reduced processing time. The result was hardened outer layer and unprocessed, soft central part because of irregular delivery of heat energy. Counter to conventional heating, this thermal effect occurs

concurrently all through the whole material being subjected to microwave exposure.[11,12]

- Enhances the penetrability of fixatives and reagents into cells
- Sustains antigenic nature of cell components.

Microwave-assisted tissue processing has been in vogue for the last 30 years. The technique has gained escalating credit in the last decade. The enhanced recognition of microwave tissue processing has led to the manufacture of commercial microwave ovens entirely planned to assure uniform rapid tissue processing under explicitly controlled specimen temperatures. These machines in addition accurately manage on-off cycling of the heating.

Microwave oven was discovered by Percy Spencer of Raytheon in 1945.[15]The earliest commercial microwave oven emerged in 1947.

Microwave ovens tender an effectual way of heating various nonconductive materials. The microwave absorption is proportional to the water content of the material in various materials. All domestic microwave ovens and laboratory microwave processors function at 2.45 GHz (corresponding to a wavelength of 12.2 cm, or just over 4-3/4").[11,12]

Microwave devices employed in histopathology laboratory:

- Microwave devices planned and certified as medical devices
- Commercial Microwave units transformed for clinical/laboratory use.
- Household Microwave devices tailored for clinical/laboratory use.

HOUSEHOLD MICROWAVE OVENS:

Provide a cost-effective method for accelerated sample preparation but have severe limitations in terms of safety and reproducibility. The magnetrons with dissimilar warm-up time along with meagre heat-conducting properties of the tissues result in unpredictable fixation of tissues. The absence of control on the temperature rise and failure to maintain the temperature at a steady level in domestic ovens, led to the discovery of laboratory-grade microwave devices.

LABORATORY MICROWAVE DEVICES:

Laboratory-grade microwave devices are swiftly acquiring recognition.

Conventional histopathology techniques rely on unhurried permeation of solutions from the external surfaces The microwave employed in histotechniques acts on the basis that electromagnetic field produces agitation of molecules that leads to their rotary motion. This motion results

in generation of energy which is emanated as heat from inside the molecules.

When thin tissue sections are bared to microwaves, they affects the whole section instantly and concurrently, leading to swap over of solutions and expedite the reaction rates owing to internal heat. The heat produced facilitates circulation of fluids in and out of the tissue sections and blocks in a more efficient manner compared to conventional heating.

FEATURES:

- Endowed with refined systems for monitoring and controlling the energy.
- Have a power transformer to generate high voltage electricity (approximately 4000V).[20]
- Accurate temperature control by means of in-built source of modifiable temperature probe.
- Agitation to foil thermal layering.
- Manifold safety features.
- Appropriate aeration.

- Provides an even environment with a Cold Spot, an enclosed bed of circulated water that incessantly absorbs the generated Microwave energy.

- The addition of a vacuum chamber in the Microwave promotes incursion of fixatives and other solvents.

COMPARISON OF DOMESTIC MICROWAVE OVEN WITH LABORATORY MICROWAVE OVEN

Domestic and Laboratory Microwave ovens are both capable of executing most of the procedures in a routine histology laboratory. However safety, reproducibility, and quality of the sample are imperative parameters whilst selecting the best device.

Household microwave ovens operate at a frequency of 2.45 GHz for the reason that it is the frequency at which polar molecules, react robustly and the microwaves sustain superior potency even at great depth.[22] This aptitude is indispensable for food preparation and is also handy for histology laboratory work. Domestic microwave oven is relatively cost-effective and produces nearly equivalent results when compared to laboratory oven.

While the laboratory ovens are auto programmed for varied processes, calibration is crucial in domestic ovens for optimal outputs.

Therefore temperature probe precision, duration of cycle time and net power levels at an assortment of settings ought to be ascertained prior to routine usage.[16]In contrast to domestic microwave ovens, the laboratory microwave oven produces uniform irradiation and do not have the tendency to cause hotspots. The magnetic stirrer placed underneath is responsible for this effect.[15]

Various combustible and noxious fumes are produced throughout the steps of processing. These are not effectively removed from domestic ovens at all times. Hence they have a vulnerability to cause explosion in the setting of an insecure electrical arrangement. This is avoided in laboratory ovens due to good ventilation.

PROPERTIES OF MICROWAVES:

Microwave energy is a component of the electro-magnetic continuum, with a frequency akin to radar beams.

- Has a frequency of 2,450 megacycles per second.[17]
- Penetrates several centimeters into biological material. This energy is absorbed and converted into heat within the tissue.
- Microwave energy is an irregular electromagnetic field that changes its course 2,450,000,000 times per second.[17] Dipolar molecules, such as water,

in attendance in the field, is subjected to vacillate at this frequency and this leads to constant stir of the molecules culminating in production of heat.

FACTORS AFFECTING MICROWAVE FIXATION:

- Period of microwave exposure
- Order of microwave irradiation
- Chemical milieu in the region of the sample during the period of irradiation.

MICROWAVE FIXATION METHODS[1]:

1) Microwave irradiation in situ:

The samples are irradiated with microwaves as an effort to safeguard them devoid of the influence of a chemical fixative.

2) Fast or ultrafast principal microwave chemical fixation:

In this process, the samples are irradiated using microwaves in a chemical milieu for a diminutive duration of time such as milliseconds or seconds.

3) Microwave exposure and then chemical fixation:

The specimens are irradiated by microwaves and subsequently soaked in a fixative like formalin for a sustained period of time to enhance homogeny of fixation.

4) Initial chemical fixation followed in sequence by microwave irradiation:

This method ensures the cross linking of fixatives inside the specimen.

5) Microwave irradiation along with freeze fixation:

Leads to decreased freezing artefacts.

RECOMMENDATIONS FOR GOOD MICROWAVE FIXATION:

- Irradiation time less than 60 seconds
- Final irradiation temperature : 50 to 55°C
- Volume of the solution is less than 50 ml in containers with at least one dimension that is ~1 cm .[28]

APPLICATIONS OF MICROWAVES IN HISTOPATHOLOGY:

Microwaves have been applied in almost all techniques in histopathology:

- Fixation

- Tissue processing
- Staining
- Frozen techniques
- Immunotechniques
- Electron microscopy
- Procedures for antigen retrieval.

Microwave-Stimulated Fixation with Fixatives:

'Fixation' is the terminology employed when chemical fixatives are used, and 'stabilization' if just physical methods of microwave heating are used. When a blend of physical effects and chemical fixatives are employed, then the phrase "microwave-stimulated fixation" has been used.[39]Microwave exposure can be used to enhance diffusion of fixation reagents into the tissue, and to accelerate the chemical process by which the fixative cross-links with the proteins of the tissue.

The most common histological fixative, formalin, is a solution composed of methylene glycol and a little amount of formaldehyde.

Normal formalin fixation occurs in three steps[41,42]

1. The methylene glycol rapidly diffuses into tissues.

2. Part of the methylene glycol is gradually transformed to formaldehyde by means of dehydration.

3. Formaldehyde bonds unhurriedly to the proteins in the tissue by cross-linking.

All the three steps can be hastened by microwave exposure.

The morphological preservation of various tissues depends on generation of an optimal temperature for each tissue, ranging between 70°C and 85°C. [29]Heating above or below these temperatures creates various artefacts such as vacuolation and changes in chromatin pattern. Excellent fume extraction is a necessity when using formalin, as fumes are highly unpleasant and great care must be taken when handling the heated formalin.

- George Bernard in his study noticed that the optimal temperature of fixation was different for different tissues. [36]
- The fixation time ought to be minimum to avert removal and displacement of diffusible ions. The duration of the microwave fixation was 1 minute. This is to a large extent earlier than most of the conventional methods.[33]

- Login GR et al.[32] shown that tissues irradiated to 50± 5˚C were found to be of better quality than those irradiated at low temperature.

- Various studies have proved that normal saline provided enhanced fixation results than formalin in microwave irradiation.[33,34,35]
- Recently new glyoxal-based fixatives that do not evaporate, even at raised temperatures, have been tried in microwave fixation.[15]
- Hsu et al[29] found that conservation of genomic and viral DNAs employed in nucleic acid hybridization analysis was of better-quality to formalin in microwave fixation using phosphate-buffered saline.

APPLICATION OF MICROWAVES IN THE PROCESSING OF TISSUES:

Tissue processing performed permits sectioning of tissue into thin sections so as to be visualized microscopically. This consists of a series of steps wherein tissues pass through various reagents, which will finally permit sectioning.[13,20]

Diffusion of reagents can be increased by the application of heat, which in turn, reduces the time.[12]Microwave histoprocessing relies on the principle of using microwave energy to speed up the process of the diffusion of liquids into and out of the specimens. The property of microwave heating will dictate the choice of processing fluids to use.

Various substances exposed to the identical amount of microwave energy heat up at dissimilar rates. The substances that heat up first have non-symmetrical polar molecules, that are effortlessly rotated by microwaves.

In contrary to conventional tissue processors with a graded series of alcohols, a clearing agent such as xylene and paraffin wax in an overnight process, microwave histoprocessing deals with only three reagents in four steps. The process consists of single change of ethyl alcohol and isopropyl alcohol and two changes in paraffin.[16]

1. 100% Ethyl alcohol for dehydration.

2. Isopropyl alcohol as the intermedium.

3. Liquid paraffin for permeation.

Clearing solutions are not necessary because the alcohol is evaporated from the tissue prior to paraffin infiltration.[22]Safety is enhanced by removing formalin and xylene from the processing method. Paraffin must be added to the microwave in liquid form as microwave energy will not melt paraffin pellets.[13]

Ralph rohr et al used two microwave tissue processing schedules.[30]

1. Short Schedule:

15 min –for small biopsies <2mm thick and <10mm in diameter.

2. Long Schedule:

60 min-for specimens>2mm thick and those with excessive blood or mucus or both.

- C.P.Mayers engaged a Microwave generator Mullard JP2-02 magnetron with a Power input 630 watts, Power output 3-200 watts in two power ranges and an operating frequency of 2,450 ± 50 megacycles/second. The tissues were trimmed to standard 1-0 cm cubes. 90 seconds pulse with 75 watts output was ideal for 1 cm cubes of tissue.[17]
- Nangia et al.,[40] in their study used fixed tissue samples that were washed under running tap water to remove formalin. Following this, the tissues were shifted to a microwave safe glass beaker consisting of 300 mL of 100% ethanol and irradiated for two cycles of 10 min each at a power of 30%. Later the tissues were placed in a beaker containing 300 mL of 100% isopropyl alcohol and microwaved for two cycles of 10 min each at 50% power for dehydration. The tissues were then moved to a beaker with 300 mL of molten paraffin wax pre-heated at a temperature of 70°C for two cycles at 50% power for impregnation.
- Dr.Anthony S.-Y. Leong has described a technique of processing 30,000 biopsies per year, by introducing a microwave stabilization stage. This

technique comprises of microwaving 20 blocks of tissue in cassettes, kept in a beaker of 500 ml normal saline on a rotator at 68˚C for 5 minutes. The stabilized blocks are then moved to a tissue processor for the rest of the processing. The lack of toxic formalin vapours in the processing area is a noteworthy advantage of this method.

- Vincent R Klump, in his work on microwave processing, washed specimens for 5 minutes in running water. 100% ethanol was used for dehydration at 65ºC for 15 minutes in microwave. Clearing was done by 100% isopropanol at 74ºC for 10 minutes and infiltrated in liquid paraffin thrice, 5 mins each time at 65ºC, 74ºC and 82º C.

- Shrestha G et al employed semi-automated laboratory grade microwave (BP¬110 Laboratory Microwave). Time needed for exchange of reagents amid steps was reduced to a minimum. Predetermined temperature was fixed for every reagent to ensure homogeny among all batches. Isopropanol: 25˚C, Ethanol: 30˚C, Molten wax: 75˚C. The mean end-temperature following dehydration step was 63.53˚C (maximum 71˚C and minimum 55˚C) and subsequent to clearing step was 75.53˚C (maximum 78˚C and minimum 73˚C).

- Meenakshi Tripathi et al[31] employed enzyme retriever V.2.2 microwave provided with a temperature probe accurate to +/- 1ºC and micro waved

500ml PBS 0.1M, pH 7.4 in a microwave safe container at 68ºC for 5 minutes.

- Prasad G Kango and RS Deshmukh used:

  Absolute alcohol: 15 minutes

  Chloroform: 15 minutes

  Paraffin impregnation: 15 minutes for microwave processing using domestic microwave. The Temperature was in the range of 45°C-58°C.

- In a study by Kok and Boon, the total processing time was 111 minutes wherein 30 blocks were prepared with an operational temperature of 75°C that was maintained in all the steps.[14]

- According to Boon et al.,[24] Chaudhari et al.,[25]andMorales et al.,[23] the tissue architecture, cell and nuclear morphology, stroma & secretory products were the same in traditionally processed and microwave processed tissue with the exception that the microwave processed tissue exhibited brighter staining with eosin and a vigorous response with haematoxylin.

- Boon et al., found that epithelium was of superior grade in microwave-processed tissues, than in traditional processing. Nevertheless, the stroma displayed focal condensation.[24]

- Panja et al., observed that microwave processed tissues exhibited considerably decreased amount of shrinkage as opposed to conventional processing. On the other hand, Kok et al., had no noteworthy difference in the quantity of tissue shrinkage.[6]

MICROWAVE EMBEDDING USING PARAFFIN

Paraffin wax has been an age old embedding medium in histoprocessing. It provides meticulous infiltration of the specimens in its liquid form and on cooling it solidifies, with petite harm to tissues.

DIFFERENCE FROM CONVENTIONAL PROCESSING:

- Fixation is complete preceding the histoprocessing steps in the microwave oven.
- Dehydration is possible in one step, as an alternative to the 2-6 steps employed in conventional processing. Use of a graded series of alcohols is not obligatory in the microwave method.
- Isopropanol can replace xylene as a clearing agent, and one bath is adequate.
- Elevated temperatures are necessary for paraffin wax impregnation.

ADVANTAGES OF MICROWAVE PROCESSING:

- The Microwave fixation method significantly decreases the processing time with single step dehydration and clearing previous to paraffin permeation.

- Microwave assisted tissue fixation removes the use of noxious and potentially toxic formalin that decreases the turnaround time and provides a workforce friendly workflow.
- Microwave irradiation avoids the requirement for xylene in tissue processing.
- It decreases long-term fixation artefacts like extraction of cellular components, and has no crystallization problems.[33]
- The decreased contact with cross-linking chemicals makes it an impressive substitute for fixation of tissues for ultra structural and genetic studies.
- Nominal amount of reagents were needed which lead to reduced cost.
- The swiftness with which microwaves can fix both large and small biopsy specimens is a key advantage.
- It largely decreases the time from tissue reception to diagnosis. This reduced procedure time allows same-day tissue processing and diagnosis without compromising the quality of the sections on the whole.
- Dependence on frozen section with its accompanying difficulties of analysis and higher expense can be reduced to a large extent.
- The averting of frozen sections also brings down the anaesthesia time.
- Critical biopsies for transplant patients can be done and diagnosed

the same day, a benefit prior to long weekends, since the overtime costs for histotechnologists and pathologists can be minimised.

- Enhances the swiftness with which neoplastic diseases are diagnosed and treatment is started.

FOR THE TECHNICIAN:

- Improved workload distribution
- Flexibility
- Can process 55-110 cassettes at a sitting
- Ease of sectioning

FOR THE PATHOLOGIST:

- Same-day diagnosis of specimens is promising.
- Strengthens the role of pathologists in management of patients.

FOR THE LAB ADMINISTRATOR:

- Enhanced work milieu for laboratory recruits
- Low cost for storage and removal of reagents

FOR THE CLINICIAN:

- Oncologists and clinicians can counsel patients within hours, based on definitive diagnosis.
- Therapy can be started without delay

FOR THE HOSPITAL ADMINISTRATOR:

- Same day results leads to reduced patient anxiety and stress
- Spectacular improvements in competence and laboratory efficiency

FOR THE PATIENT:

- Abolition of unwanted stress while awaiting a diagnosis
- Appropriate start of necessary therapy without delay

DISADVANTAGES:

- Needs adequate calibration and monitoring.
- Temperatures ought to be maintained between 70˚c and 85˚c.
- The tissue section should not be more than one cubic centimeter while fixed, or else incomplete penetration of microwaves will occur.
- Tissue shrinkage.

- Tissue sponginess.
- Red blood cell lysis.
- Conventional processing results in peak laboratory activity in the mornings, while microwave histoprocessing produces a continuous and even activity all through the day, which may not be acknowledged by the laboratory personnel.

HAZARDS :

The use of microwave ovens for simple heating or defrosting in laboratories can pose a number of hazards, including:

- Ignition of flammable vapours
- Electric shock from ungrounded or faulty units
- Ignition of materials being heated
- Pressure build-up in sealed containers
- Sudden boiling of liquid in an open container following removal from an oven.
- Exposure to microwave radiation from a faulty or modified unit.

Australian radiation protection and nuclear safety guidelines provide limits on the microwave leakage during use to <50W/m2 at 50 mm from any oven surface.

The following protocol has to be followed in the operation of microwaves. The oven cavity should be sufficiently ventilated. The unit

ought to be positioned on a clear open bench and not in a setting where the vents could be blocked by books or paraphernalia. Customary inspections have to be made to ensure that the sealing surfaces are clean and do not show any indication of damage. Microwave leakage has to be thought of in the occurrence of arcing or burn marks.

The microwave ovens ought to be electrically grounded and linked using a appropriately rated three-pin cord and plug. As for any fresh laboratory equipment, microwave ovens have to be scrutinized in accordance with the guidelines for electrical equipment to ascertain agreement with this requisite. The defects in equipment or hitches in operation with a microwave oven have to be promptly identified and reported without delay to the laboratory supervisor or overseer.

Wherever possible microwave grade plastic vessels ought to be used with a pressure relief valve. When glass vessels are used test them out for cracks and flaws on every occasion prior to placing in the microwave. Proper shielding equipments have to be employed while taking out heated liquids from the oven.

No attempt must be made to heat flammable liquids or solids, hazardous substances or radioactive materials in any type of microwave oven, whether domestic or laboratory-grade. We should not override the interlock switches that prevent a microwave oven from operating with the door open. Wires, cables, tubing etc must not be placed between the door

and the seal. The mechanical or electrical systems of a microwave oven should not be modified in any way.

Whenever a unit is suspected to be faulty it should be disconnected from the power supply, removed from service and labelled with an appropriate tag while awaiting repair or disposal. Any irreparable or redundant microwave oven should be rendered inoperable by removal of the plug and cord, before disposal.

Microwave ovens in a laboratory have to be averted from preparation of food. Sealed containers must not be heated in a microwave oven. Even a loosened cap or lid poses a noteworthy risk since microwave ovens can heat material so quickly that the lid can seat upward against the threads and containers can explode either in the oven or shortly after removal.

Metal objects of any kind including aluminium foil and plastic coated magnetic stirrer bars have to be avoided in a microwave oven. Overheat liquids in a microwave oven ought to be refrained from as it is possible to raise water to a temperature greater than the normal boiling point; when this occurs, any disturbance to the liquid can trigger violent boiling that could result in severe burns.

Microwave tissue fixation and processing create sections of comparable or even enhanced quality to that produced by conventional processing. This has resulted in significant curtailing of turnaround times, enabling same-day diagnosis facilitating patient diagnosis and management on a single

day basis. This progress in turnaround time help in decreasing costs associated with diagnosis and averting patient discomfort and increases the swiftness with which neoplastic diseases are diagnosed and with an early initiation of treatment.

MATERIALS AND METHODS

The study was conducted in the Department of Pathology, Tirunelveli Medical College and Hospital, Tirunelveli. It was done after obtaining the necessary approval from Institutional Ethical Committee of Tirunelveli Medical College, Tirunelveli.

STUDY DESIGN:

Experimental study.

STUDY SITE:

Tissue materials sent for histopathological examination from Tirunelveli medical college hospital to the Department of Pathology, Tirunelveli Medical College, Tirunelveli were handled in this study.

SOURCE:

Unfixed tissue slices from various histopathological specimens.

SAMPLE SIZE:

100 cases using formaldehyde and a new (minimal formalin containing) compound fixative.

DURATION OF STUDY:

October 2015 to June 2017.

INCLUSION CRITERIA:

Tissue materials received from the operation theatres of Tirunelveli medical college hospital.

Portion of the tissue materials were used in the study.

EXCLUSION CRITERIA:

- Autolysed specimens
- Tissues fixed in other fixatives.

MATERIALS REQUIRED:

- Concentrated formalin
- Absolute ethyl alcohol
- Glycerin
- Methylene blue
- Distilled water
- Sodium chloride
- Sodium Dihydrogen Phosphate Monohydrate
- Anhydrous disodium hydrogen phosphate

METHODOLOGY:

New compound fixative was prepared in our department using formalin, ethanol, glycerin and hypotonic saline.[9]

Cell shrinkage may be caused by Ethanol. This can be avoided by reconstituting in hypotonic saline. Evaporation was curtailed by addition of Glycerin. Addition of methylene blue gave a light blue color to the fixative. This helped to scrutinize the color of fixatives and to shun the inclination to smell the components.

Multiple human tissue materials from varying sites (skin, viscera, lymph nodes, bones etc.,) were utilized. Fixation was done in formalin and new compound fixative. Unfixed raw tissue slices were immediately fixed in the prepared compound fixative after collecting from MOT and GOT. Fixation time was titrated for both formalin & the new compound fixative.

NEW COMPOUND FIXATIVE:

COMPOSITION[9]

- Formalin – 6%
- Ethanol - 30%
- Glycerin - 5%
- Methylene Blue- 0.05%
- 0.7% hypotonic saline

Buffer:

- Sodium dihydrogen phosphate monohydrate - 4g
- Anhydrous disodium hydrogen phosphate - 6g

0.7% hypotonic saline was used to make up the volume to 100 ml. The ph was maintained between 7. 2 - 7.4.

Tissue samples were taken from various specimens and they were categorized into

- Small biopsies
- Soft
- Intermediate
- Hard
- Decalcified tissues.

Two representative bits from each specimen was taken. The unfixed tissues were placed in plastic cassettes. These cassettes were then immersed in coplin jars, one containing formalin and the other with new compound fixative.

Fixation of tissues in Formalin & new compound fixative solutions were done using microwave oven at 50˚c for 90 seconds, two pulses.

PROTOCOL FOR TISSUE PROCESSING:[30]

The tissues were washed thoroughly in water.

Methanol - 7 Minutes at 40˚c

Methanol - 7 Minutes at 40˚c

Isopropyl Alcohol - 8 Minutes at 40˚c

Isopropyl Alcohol - 8 Minutes at 40˚c

Xylene - 10 Minutes

Wax I - 30 Minutes at 75˚c

Wax II -30 Minutes at 75˚c.

In our study we used xylene for better clearing which is not routinely used in microwave processing. Embedding of processed tissues were done using paraffin wax and 4 micron thickness sections were taken by means of a microtome. Staining was done by routine hematoxylin and eosin staining.

STAINING PROCEDURE:

HEMATOXYLIN SOLUTION PREPARATION:[5]

Hematoxylin - 2.5 g

Potassium alum - 50 g

Sodium iodate - 0.5 g

Absolute ethanol - 25 cc

Glacial acetic acid - 20 cc

Distilled water- 500cc

Hematoxylin is initially dissolved in absolute alcohol, and then alum is added to it. The alum ought to be primarily dissolved in warm distilled water. The mixture is allowed to boil followed by careful addition of sodium iodate. The stain is quickly cooled with subsequent addition of acetic acid and the resultant stain can be used immediately.

EOSIN PREPARATION[5]

Eosin Y - 1 g

95% ethanol - 80 cc

Glacial acetic acid - 0.2 cc

Distilled water - 20 cc

Eosin Y is first dissolved in distilled water followed by subsequent addition of 95% ethanol and glacial acetic acid.

STAINING:

1. Xylene 3 changes – 2 minutes each.
2. Graded alcohols (90%, 80%, 70%) – 10 dips each.
3. Expose sections to water.
4. Harris hematoxylin – 15 minutes.
5. Wash in clean water.
6. Differentiate by 1% acid alcohol.
7. Wash in clean water.
8. Bluing using 0.5% lithium carbonate.
9. Rinse in clean water.

10. Eosin – 15 seconds to 2 minutes based on the age of eosin.
11. Wash in clean water.
12. Dehydration in absolute alcohol - two changes,10 dips each.
13. Xylene – two changes, 10 dips each.
14. Mount the slides using DPX mountant.

Stained slides were observed using light microscope and analysed by two independent pathologists. Cellular Architecture, Cytoplasmic, Nuclear details Staining characteristics and Fixation artifacts were studied.

A scoring system based on the Nuclear, cytoplasmic and architectural features were followed. Score 3 was given to nuclear, cytoplasmic and architectural features of all tissues fixed in new compound fixative that are comparable to those fixed in 10% NBF.

NUCLEAR FEATURES:

The following nuclear parameters were assessed:

- Preservation of nucleus and nucleolus
- Nuclear size
- Regularity of nuclear membrane

Score 3 was assigned to sections fixed in new compound fixative with comparable nuclear parameters to sections that were fixed in 10% NBF. Score 2 was assigned to tissues having 1 to 2 less defined nuclear

details. Score 1 was assigned to tissues having more than 2 less defined nuclear features. Score 0 was assigned to sections with poor conservation of details, not suitable for diagnosis.

CYTOPLASMIC FEATURES:

Assessment was done by considering the following cytoplasmic features.

- Color of cytoplasm
- Volume
- Nuclear cytoplasmic contrast
- Erythrocyte integrity.

Score 3 was assigned to tissues fixed in new compound fixative with comparable cytoplasmic details to tissues fixed in 10% NBF. Score 2 was assigned to sections exhibiting cytoplasmic shrinkage. Score 1 was assigned to sections having greater than two less specific cytoplasmic details. Score 0 was assigned to sections with poor conservation of features not suitable for diagnosis.

ARCHITECTURAL FEATURES:

Assessment was based on the following architectural features:

- Shrinkage artifacts
- Cracking

- Formalin pigments.

Score 3 was assigned to tissues fixed in compound fixatives with comparable architectural features to tissues that were fixed in conventional 10% NBF. Score 2 was assigned to sections having one to two ill defined architectural features. Score 1 was assigned to sections having more than two ill defined architectural details. Score 0 was assigned to sections with poor conservation of features, not suitable for diagnosis.

The microwave processed tissues fixed in new compound fixative was evaluated and compared with formalin fixation. Tabulation of results were done. Chisquare test, Z score test and Fisher exact test were used for statistical analysis of differences between formalin fixed tissues and specimens fixed in new compound fixative. p -values less than 0.05 were considered significant.

OBSERVATION AND RESULTS

A total of 100 specimens were included in this study. Tissues from various systems were taken into consideration in this study. They were categorized into Soft, Intermediate, Hard, Small biopsy and Decalcified tissue. One bit was placed in formalin and another in new compound fixative.

A domestic microwave was used. Initially, the tissues were fixed at 60˚c for 90 seconds in 2 pulses. Mild boiling of tissues was observed. Alcoholic fumes were present. Hence fixation temperature was titrated to 50˚c for 90 seconds in 2 pulses.

TABLE 1: CATEGORY OF SPECIMENS

Specimen	**No. of cases(n=100)**	**Percentage**
Small biopsy	20	20
Soft	6	6
Intermediate	55	55
Hard	16	16
Decalcified	3	3

CHART 1: CATEGORY OF SPECIMENS

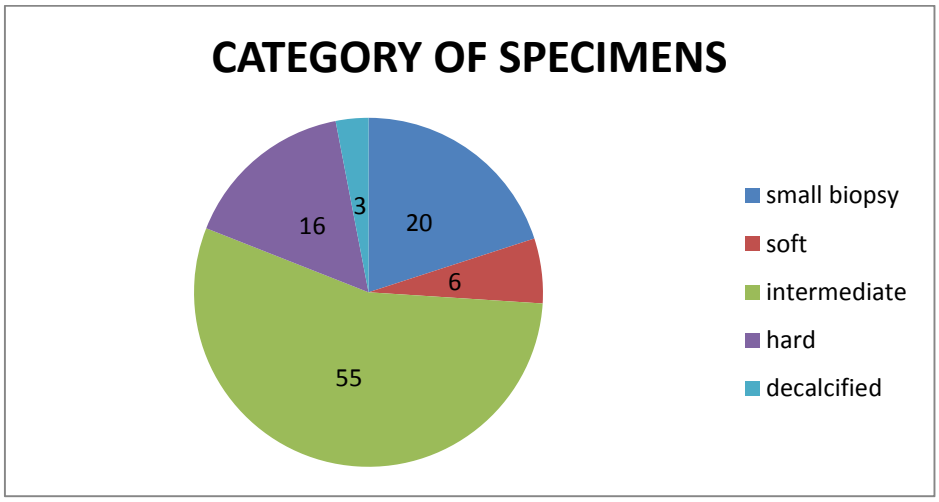
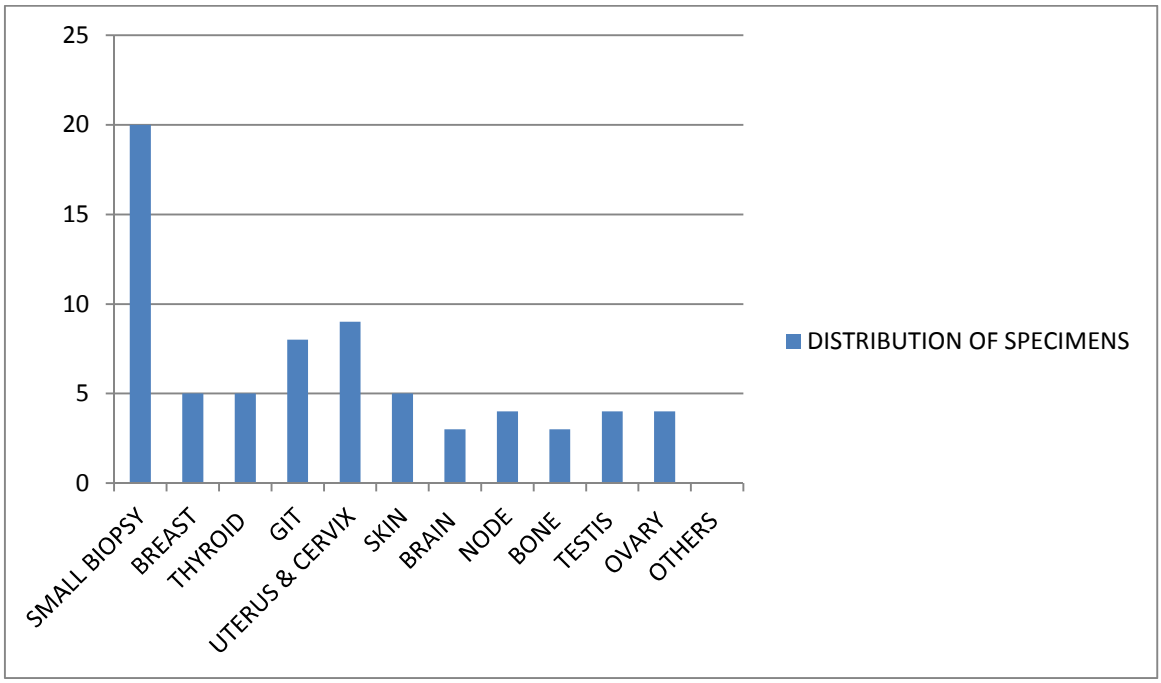


TABLE 2 : DISTRIBUTION OF SPECIMENS

Specimen	No. of cases(n=100)	Percentage
SMALL BIOPSY	20	20
BREAST	5	5
THYROID	5	5
GIT	8	8
UTERUS & CERVIX	9	9
SKIN	5	5
BRAIN	3	3
LYMPH NODE	4	4
BONE	3	3
TESTIS	4	4
OVARY	4	4
OTHERS	30	30

CHART 2: DISTRIBUTION OF SPECIMENS



GROSS APPEARANCE:

- Consistency of tissues after fixation in new compound fixative was same as tissues fixed in 10% NBF.
- No cutting and sectioning difficulties were encountered.

ANALYSIS OF HISTOMORPHOLOGICAL CHARACTERISTICS:

Stained slides were analysed by two independent pathologists using light microscope. Cellular Architecture, Cytoplasmic, Nuclear details, Staining characteristics and Fixation artifacts were studied.

COMPARISON OF NUCLEAR CHARACTERISTICS:

The following nuclear parameters were assessed:

- Nuclear size
- Preservation of nucleus and nucleolus
- Regularity of nuclear membrane

Score 3 was assigned to sections fixed in new compound fixative with comparable nuclear parameters to sections that were fixed in 10% NBF. Score 2 was assigned to tissues having 1 to 2 less defined nuclear details. Score 1 was assigned to tissues having more than 2 less defined nuclear features. Score 0 was assigned to sections with poor conservation of details, not suitable for diagnosis.

COMPARING NUCLEAR FEATURES OF NEW COMPOUND FIXATIVE AND 10% NBF:

Nuclear features of tissues fixed in new compound fixative were compared with formalin fixed tissues, both being subjected to microwave fixation and processing. Out of the 100 specimens, 97 specimens presented with nuclear features comparable to formalin and scored 3. Three of the specimens especially the decalcified tissues showed nuclear shrinkage compared to 10% NBF and hence received a score of 2.

CHART 3: NUCLEAR FEATURES OF NEW COMPOUND FIXATIVE VS 10% NBF

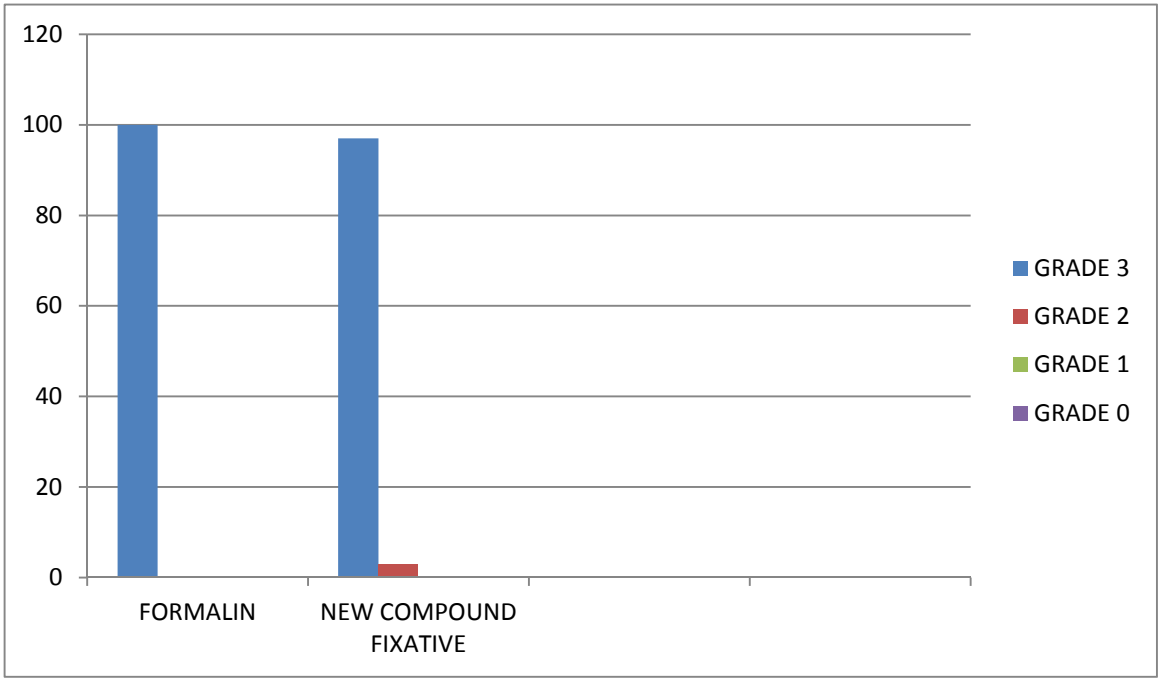


TABLE 3: COMPARING NUCLEAR FEATURES OF NEW COMPOUND FIXATIVE AND 10% NBF:

FIXATIVES	NO.OF CASES WITH VARYING SCORES				p value*
	3	2	1	0	
FORMALIN	100	Nil	Nil	Nil	**n/a**
NEW COMPOUND FIXATIVE	97	3	Nil	Nil	**0.080**

*** CHI SQUARE TEST**

The above table shows that the p value is 0.080, which is not significant, thereby showing that there is no significant difference between 10% NBF and New compound fixative. Hence the new compound fixative is equivalent to 10% NBF in preserving nuclear features.

COMPARISON OF CYTOPLASMIC FEATURES:

Cytoplasmic features that were assessed:

- Color
- Volume
- Nuclear cytoplasmic contrast
- Erythrocyte integrity**.**

Score 3 was assigned to cytoplasmic features of sections fixed in new compound fixative that were comparable to those fixed in 10% NBF. Score 2 was assigned to sections with cytoplasmic shrinkage. Score 1 was assigned to tissues having more than 2 less defined characteristics. Score 0 was assigned to sections with poor conservation of details, not suitable for diagnosis.

COMPARISON OF CYTOPLASMIC CHARACTERISTICS OF NEW COMPOUND FIXATIVE AND 10% NBF:

In this chart and table, cytoplasmic features were compared between new compound fixative and formalin. Of the total specimens fixed in new compound fixative, 97 have been assigned score 3 as they exhibited features akin to sections fixed in 10% NBF. Remaining three cases were assigned score 2 as they exhibited cytoplasmic shrinkage and poor nuclear cytoplasmic contrast.

CHART 4: CYTOPLASMIC FEATURES OF NEW COMPOUND FIXATIVE VS 10% NBF

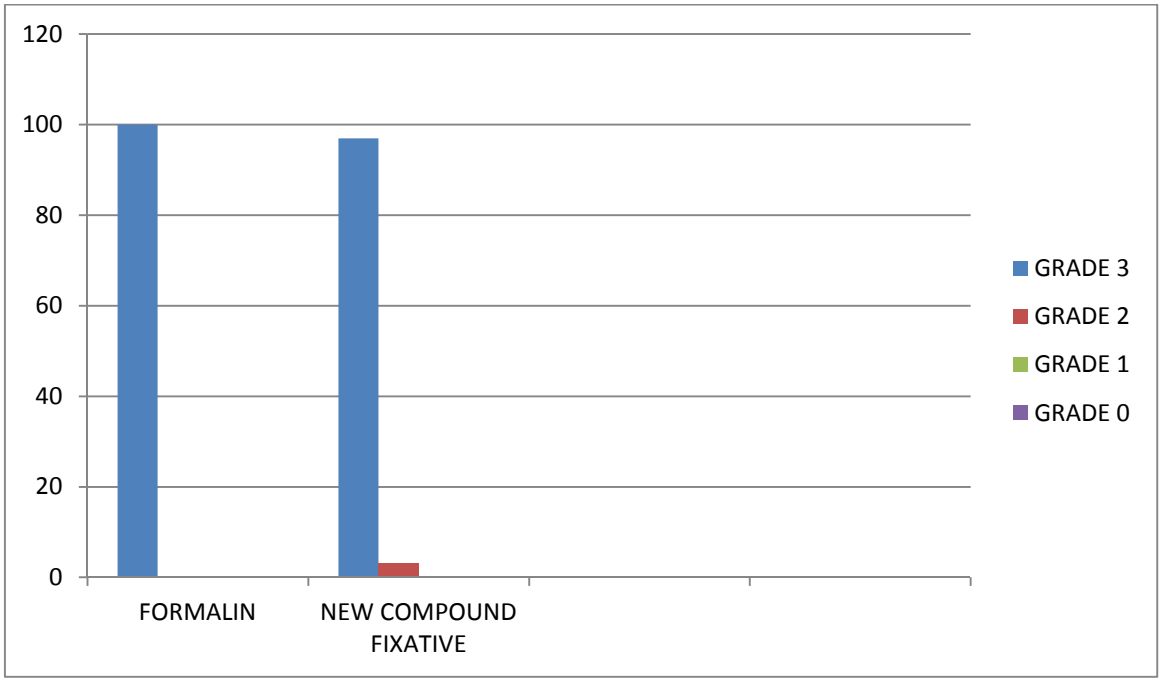


TABLE 4: COMPARISON OF CYTOPLASMIC CHARACTERISTICS:

FIXATIVES	**NO.OF CASES WITH VARYING SCORES**				**p value***
	3	**2**	**1**	**0**	
FORMALIN	100	Nil	Nil	Nil	**n/a**
NEW COMPOUND FIXATIVE	97	3	Nil	Nil	**0.080**

***CHI SQUARE TEST**

The above table shows that the p value is 0.080, which is not significant, thereby showing that there is no significant difference between 10% NBF and New compound fixative. Hence the new compound fixative is equivalent to 10% NBF in preserving the cytoplasmic characteristics.

COMPARISON OF ARCHITECTURAL FEATURES:

Architectural features were assessed based on:

- Shrinkage artifacts
- Cracking
- Formalin pigments

Score 3 was assigned to architectural features of tissues fixed in new compound fixative that were comparable to those fixed in 10% NBF. Score 2 was given to sections with shrinkage artefacts, distortion and cracking. Score 1 was assigned to tissues having more than 2 poorly defined features. Score 0 was assigned to sections with poor conservation of details, not suitable for diagnosis.

COMPARISON OF ARCHITECTURAL FEATURES OF NEW COMPOUND FIXATIVE AND 10% NBF:

Architectural features were compared between new compound fixative and 10% NBF. 93 of the sections fixed in new compound fixative scored three. Decalcified sections got a score of 2 due to shrinkage artefact and distortion. Nodal tissues also got a score of 2 due to narrowing of subcapsular and interfollicular sinuses.

CHART 5: ARCHITECTURAL FEATURES OF NEW COMPOUND FIXATIVE VS 10% NBF

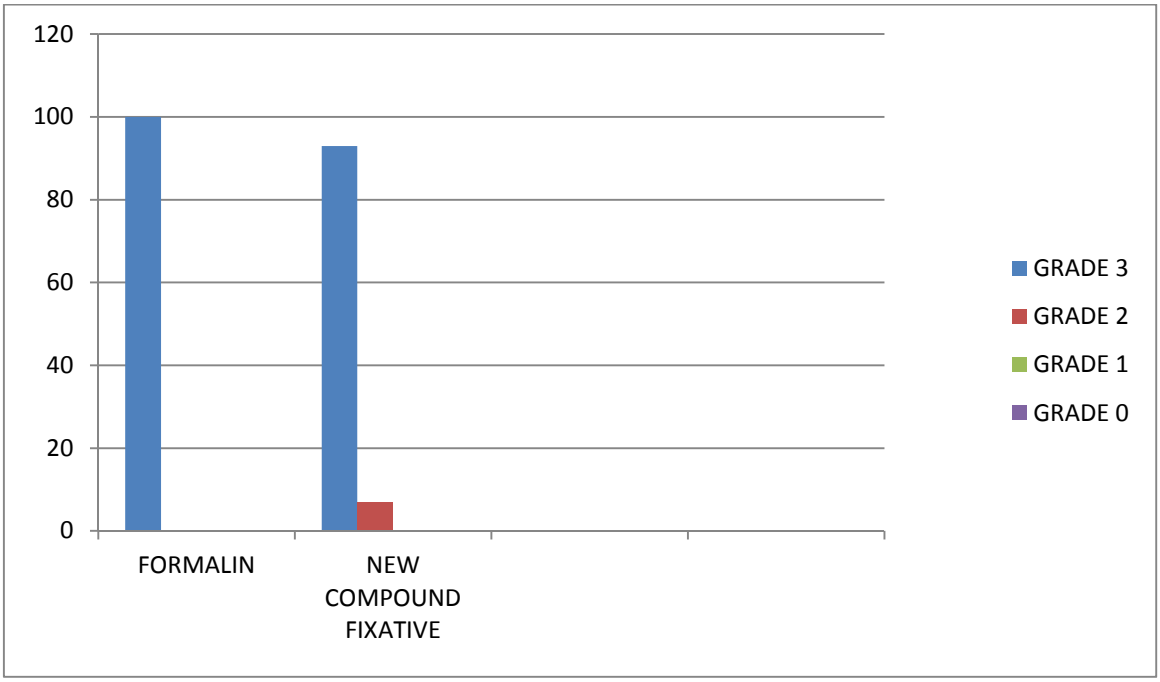


TABLE 5: COMPARISON OF ARCHITECTURAL CHARACTERISTICS:

FIXATIVES	**NO.OF CASES WITH VARYING SCORES**				**p value***
	3	**2**	**1**	**0**	
FORMALIN	100	Nil	Nil	Nil	**n/a**
NEW COMPOUND FIXATIVE	93	7	Nil	Nil	**.007**

***CHI SQUARE TEST**

The above table shows there is no significant difference between new compound fixative and formalin. The p value is significant. So both fixatives are equal in architectural detail preservation.

In our study, consistency of tissues after fixation in new compound fixative was same as tissues fixed in 10% NBF.

The overall fixation time was decreased from conventional 12 hours to 3 minutes while using microwave fixation. The total processing time

was reduced from twelve hours to one hour and forty five minutes. For fatty tissues it was further reduced to twenty six minutes followed by wax embedding for one hour.

On microscopic examination by two independent pathologists, the inter-observer variation was found to be minimal. The z score test and fisher exact test were done and the scrutiny revealed that both techniques were showing the similar cellular morphology. The results were statistically insignificant. The cellular outline, cytoplasmic detail, nuclear detail, erythrocyte integrity as seen after after fixation in new compound fixative were same as tissues fixed in 10% NBF.

Partial tissue loss was noted in adipose tissues. This was not seen when the microwave processing time was reduced from 7 minutes in methanol to 5 minutes and 8 minutes in iso propyl alcohol to 6 minutes.

The cellular outline and cytoplasmic details of the tissues fixed in new compound fixative and microwave processed was comparable to formalin fixed tissues. However, in our study cell crowding and overlapping were minimal and the nuclear features such as vesicular nucleus, prominent nucleolus and the presence of mitoses were brought out well in tissues fixed in new compound fixative.

Staining of the microwave fixed tissues was sharper and brighter in most of the tissues owing to a brighter staining with eosin and a stronger reaction with haematoxylin. The nuclear staining was more accentuated in tissues obtained after fixation in new compound fixative.

Erythrocyte integrity was maintained in the microwave irradiated tissues. In both methods of fixation, inflammatory cells like lymphoctyes and plasma cells were discernible from one another.

Small biopsies such as oropharyngeal tissues were morphologically better when fixed in new compound fixative in microwave oven.

Adipose tissue showed the best morphology and staining following fixation in new compound fixative. Mature fat cells were clearly delineated.

Testicular tissue fixed in new compound fixative displayed superior accentuation of nuclear configuration compared to formalin fixed counterparts. The meiotic divisions were well made out. The different cells such as primary, secondary spermatocytes, spermatids, sertoli and leydig cells were very well differentiated.

Keratin in the squamous epithelium of skin is quite difficult to stain in formalin fixed skin, while specimens fixed by new compound fixative using microwave showed accentuation of keratinised layer by eosin.

Decalcified tissues such as bone fixed using new compound fixative displayed hardening of tissues, cutting difficulties, thick sections and shrinkage artifacts.

Hyalinisation is quite common in leiomyomas. The hyalinised areas in specimens fixed in new compound fixative tend to take up a lighter shade of eosin as compared to those fixed in formalin.

Neoplastic tissues in our study were well preserved in the same way as the non-neoplastic microwave fixed tissues .Features of dyskeratosis were well made out in tissues fixed in new compound fixative. In intestinal biopsies, mitosis towards the lumen is diagnostic, which is prominent in tissues fixed in new compound fixative. This is quite useful in cases where specimen is inadequate.

Epithelial Interface and connective tissue have been a special area of interest in ruling out invasion. This is well made in microwave fixed tissues. We also noted eosinophilia of sub epithelial collagen.

DISCUSSION

Quick processing of histopathological tissues is an imperative need of the hour to accomplish the requirements of clinicians attending to intensely ailing patients. Turn-around time is a vital concern in tissue processing. At first, steps were taken to reduce the tissue processing time for intra-operative frozen sections and specimens where a swift diagnosis and quick commencement of treatment based on the histopathology findings were required. In this era of decreased health care costs and modern diagnostic procedures, it is imperative to reduce the turn-around time for routine histopathological specimens as well.

Pathology laboratories employ enormous amount of formalin without adequate thought on its noxious side effects. In the yester years, search has been made for harmless substitutes to take the place of formalin. This has not been successfully accomplished owing to parameters such as structural and antigenic alterations.[62] Therefore it is imperative to intensify the lookout for alternatives to formalin fixation.

In our study, we have used the new compound fixative that was prepared in our department. The fixation characteristics of the new compound fixative has been studied earlier[9] and was found to be

comparable to conventional formalin fixed tissues. The other features such as its application in microwave fixation and its role in special stains have not been determined completely. In this study, we have tried to evaluate the role of the new compound fixative in microwave fixation by analyzing the cytomorphological features of tissues fixed in minimal formalin containing compound fixatives.

An attempt has also been made to decrease the formalin concentration thereby diminishing the contact with formalin among technicians handling the histopathological specimens. We have also used microwaves for fixation and processing in order to decrease the specimen output time.

Domestic microwave oven has been used due to cost constraints, as this is an experimental study and also due to the fact that the end result was as good as that produced by laboratory grade microwave ovens.

Cathy.B.Moelans et al found that tissues fixed in Finefix and RCL2 were paler compared to specimens fixed with NBF.[60]However Cristina Zanini et al in their study found that specimens fixed in PAGA, ZBF and RCL2 had no color change[52]. In the current study, consistency of tissues after fixation in new compound fixative was same as tissues fixed in 10% NBF. Moreover, odour of the new compound fixative was found to be less irritant than formalin.

C.P.Mayers[19] observed that the optimum energy level and duration of exposure suitable for 1 cm cubes of tissue was 75 watts output for 90 seconds. Initially, the tissues were fixed at 60˚c for 90 seconds in 2 pulses in our study. Mild boiling of tissues was observed. Alcoholic fumes were present. Hence fixation temperature was titrated to 50˚c for 90 seconds in 2 pulses.

Fixation time was reduced from hours to few minutes, which was noted in many studies.[25,35,47,48,49]In the present study, the overall fixation time was decreased from conventional 12 hours to 3 minutes while using microwave fixation.

Microwave processing significantly reduces turn-around times.[53]The processing time excluding fixation and cutting was observed to be around one hour in the work done by Harsh kumar et al.,[55]In the work by Kok and Boon, the entire processing time was 111 minutes when 500-ml containers were employed and 30blocks were got. The working temperature of 75ºC was maintained throughout the procedure.[14]The time required for microwave processing was 80(+/- 5) minutes in the study done by V. Steri et al.[54] The total processing time was reduced from twelve hours to one hour and forty five minutes in our study. For fatty tissues it was further reduced to twenty six minutes followed by wax embedding for one hour.

On microscopic examination by two independent pathologists, the inter-observer variation was found to be minimal. The chisquare test, z score test and fisher exact test were done and the scrutiny revealed that both techniques were showing the similar cellular morphology. The results were statistically insignificant with regard to nuclear and cytoplasmic features. The cellular outline, cytoplasmic detail, nuclear detail and architectural features as seen after after fixation in new compound fixative were same as tissues fixed in 10% NBF.

Ragazzini T et al.,[43]observed that the loss of tissue in domestic microwave was due to the fact that a constant temperature cannot be attained or maintained in a specific time. In our study, partial tissue loss was noted in adipose tissues. This was not seen when the microwave processing time was reduced from 7 minutes in methanol to 5 minutes and 8 minutes in iso propyl alcohol to 6 minutes.

Kango and Deshmukh[14] and Boon et al.,[24] in their studies observed that the microwave processed epithelium showed excellent nuclear cytoplasmic contrast. Likewise our study too provided epithelium with significant nuclear cytoplasmic contrast when processed by microwave assisted tissue processing.

L. Benerini Gatta et al [61]observed that there was no difference in cytoplasmic features between alternative fixatives and formalin. The present study demonstrates that there is no significant difference between new compound fixative and 10% NBF with regard to cytoplasmic parameters. Hence both were equal in conserving cytoplasmic parameters in most of the tissues except the decalcified ones which exhibited cytoplasmic shrinkage and poor nuclear cytoplasmic contrast which earned them a score of 2.

Meenakshi Tripathi et al,[31]Kennedy A et al.,[44]and Reed W et al.,[45] found that the cytological details of the nuclei was much clearer using the microwave technique. In the study by Cristina Zanini et.al[52], nuclear features were better preserved in alcohol based fixatives. This is consistent with other studies.[14,15,31] Similarly, in our study cell crowding and overlapping were minimal and the nuclear features such as vesicular nucleus, prominent nucleolus and the presence of mitoses were brought out well in tissues fixed in new compound fixative.

The most important limitation of coagulant fixatives is the profound shrinkage of the tissue sections. Robert Bacallao et al., have observed that methanol shrinks cell height by 50% in the fixation process.[63]On comparing nuclear features tissues fixed in new compound fixative and 10% NBF, the present study indicates that there is no significant difference

between them .So both are equal in preserving nuclear features in most of the tissues except the decalcified ones which exhibited nuclear shrinkage that gave them a score of 2.

In the study by Mahdiieh Ghoddosi et al, more than 90% cases in their study got good score on the morphological features. The study by Suresh durai et al observed that lymphoreticular tissues fixed in compound fixative showed shrinkage artifacts and Thyroid specimens also showed significant retraction artifacts. Our study found that there is statistically significant difference between new compound fixative and conventional formalin with regard to conserving the architectural parameters of lymph nodes and decalcified tissues. Nodal tissues also got a score of 2 due to narrowing of subcapsular and interfollicular sinuses. Decalcified sections got a score of 2 due to shrinkage artefact and distortion.

The studies of Panja P et al., [6] Meenakshi Tripathi et al,[31] and Ragazzini T et al.,[43] showed that the staining of the microwave fixed tissues was sharper and brighter. In most of the tissues, microwave processed sections had a brighter staining with eosin and a stronger reaction with haematoxylin unlike the conventionally processed tissues. Nevertheless in our study, the nuclear staining was more accentuated in tissues obtained after microwave fixation in new compound fixative.

Erythrocyte integrity was maintained in the microwave irradiated brain sections in the studies by Boon ME et al.,[46] and Meenakshi Tripathi et al.,[31]Erythrocyte integrity was maintained in the microwave irradiated tissues in our study, which is in disparity to the studies of Mayer CP [19] and Hopwood D et al.,[47] who noticed RBC lysis .

Inflammatory cells like plasma cells and lymphoctyes were discernible from each other in the sections subjected to microwave processing, as depicted in the studies of Kango and Deshmukh[14] and Harsh kumar et al.,[55] as is also demonstrated in this current study.

Rohr LF et al.,[30] found that microwave fixed small biopsies were morphologically better. In the present study, small biopsies such as oropharyngeal tissues were morphologically better when fixed in new compound fixative in microwave oven.

Adipose tissue showed the best morphology and staining following microwave fixation in the findings of Babu TM et al.,.[50] this is similar to our study, where adipose tissue fixed in new compound fixative showed good morphological preservation. Mature fat cells were clearly delineated. Few observers like Suri V et al., [21]found that tissues containing fat responded poorly to microwave irradiation.

Testicular tissue fixed in new compound fixative displayed superior accentuation of nuclear configuration compared to formalin fixed counterparts. The meiotic divisions were well made out. The different cells such as primary, secondary spermatocytes, spermatids, sertoli and leydig cells were very well differentiated. This could play a vital role in the usage of new compound fixative in microwave processing of testicular biopsies.

Keratin in the squamous epithelium of skin is quite difficult to stain in formalin fixed skin, while specimens fixed by new compound fixative using microwave showed accentuation of keratinised layer by eosin.

The time needed for decalcification of bone by microwave processing can be decreased to 1/5–1/10 of the initial preparation time.[58] Decalcified tissues such as bone fixed using new compound fixative in our study, displayed hardening of tissues, cutting difficulties, thick sections and shrinkage artifacts.

Findings of Mathai et al.,[56] Hopwood D et al.,[47],Emerson LL et al.,[51] showed that malignant cells were well preserved in microwave assisted tissue processing. The characteristic features of malignancy like anisonucleosis, pleomorphic nuclei, prominent nucleoli, hyperchromatism and mitotic figures were akin to conventionally processed tissues. Neoplastic tissues in our study were well preserved in the same way as the non-neoplastic microwave fixed tissues .Features of dyskeratosis was well

made out in tissues fixed in new compound fixative. In intestinal biopsies, mitosis towards the lumen is diagnostic, which is prominent in tissues fixed in new compound fixative. This is quite useful in cases where specimen is inadequate.

Epithelial Interface and connective tissue have been vital parameters in ruling out invasion. This is well made in microwave fixed tissues as noted in the study by Harsh kumar et al.,[55] which showed similar tissue architecture and stroma, when compared with the conventionally processed technique. We also noted eosinophilia of sub epithelial collagen. Hyalinisation of leiomyoma is quite common. However hyalinised areas tend to take lighter shade of eosin when compared to formalin fixed tissues.

Cristina Zanini et al reported that specimens fixed in fixatives alternative to formalin were apt for microtomy.[59]Cathy.B.Moelans et al found that RCL2 fixed tissues demonstrated sectioning difficulties owing to their dicey nature.[60]Tissues fixed by new compound fixative were appropriate for microtomy in our study. There were no cutting difficulties.

SUMMARY AND CONCLUSION

The present study was done in Department of Pathology, Tirunelveli Medical College with the purpose of studying about compound fixatives in microwave assisted tissue processing, thereby trying to reduce the turn around time and establishing a formalin free work environment. The study comprised of two sets of 100 different specimens of varying tissue characteristics fixed in a new compound fixative and formalin respectively. New compound fixative was prepared from ethanol, minimal quantity of formalin, glycerin and hypotonic saline. Addition of glycerin produced minimal evaporation of ethyl alcohol which resulted in less odor and fumes in the laboratory. The tissues were fixed and processed using a domestic microwave oven.

The overall fixation time was decreased from conventional 12 hours to 3 minutes while using microwave fixation. The total processing time was reduced from twelve hours to one hour and forty five minutes which reinforced the fact that microwave processing reduces the turnaround time. For fatty tissues it was further reduced to twenty six minutes followed by wax embedding for one hour.

Architectural features were well conserved in 93% of tissues fixed in new compound fixative and were comparable to formalin fixed tissues. The remaining 7% of the cases included decalcified tissues which exhibited

shrinkage artifact and distortion and nodal tissues that presented with narrowing of subcapsular and interfollicular sinuses.

Cytoplasmic and nuclear parameters of tissues fixed in new compound fixative were well preserved and were comparable to 10% NBF. Majority (97%) of the tissues got a maximum score of three which was in par with formalin fixed tissues. A minimal number of tissues (3%) especially the decalcified category exhibited cytoplasmic and nuclear shrinkage with a poor nuclear cytoplasmic contrast.

Specimens fixed by new compound fixative using microwave showed accentuation of keratinised layer by eosin. This is quite useful in the diagnosis of dermato pathological specimens.

Neoplastic tissues in our study were well preserved in the same way as the non-neoplastic microwave fixed tissues. Features of dyskeratosis were well made out in tissues fixed in new compound fixative. Luminal mitosis in intestinal biopsies is a significant diagnostic clue. This is prominent in tissues fixed in new compound fixative and may be helpful in situations where specimen is inadequate.

In the present study, small biopsies such as oropharyngeal tissues were morphologically better when fixed in new compound fixative in

microwave oven. This feature can be used in quick processing and diagnosis of a load of small biopsies that we encounter every day.

Testicular tissues fixed in new compound fixative displayed superior accentuation of nuclear configuration compared to formalin fixed counterparts. The meiotic divisions were well made out. This could play a vital role in the usage of new compound fixative in microwave processing of testicular biopsies.

The current study demonstrates that minimal formalin containing compound fixatives can be effortlessly prepared in the histopathological laboratory. Microwave processing using minimal formalin containing compound fixative has been found to be cost effective with reduced specimen turnaround times. It also produced sections comparable to formalin fixation which establishes its efficiency and has reinforced its role in replacing formalin in histopathological laboratories. It has the added advantage of creation of a better laboratory environment owing to decreased vapor density of formalin and enhanced air quality.

FIG 1:NEW COMPOUND FIXATIVE AND FORMALIN

FIG 2: DOMESTIC MICROWAVE OVEN

FIG 3:MICROWAVE FIXATION

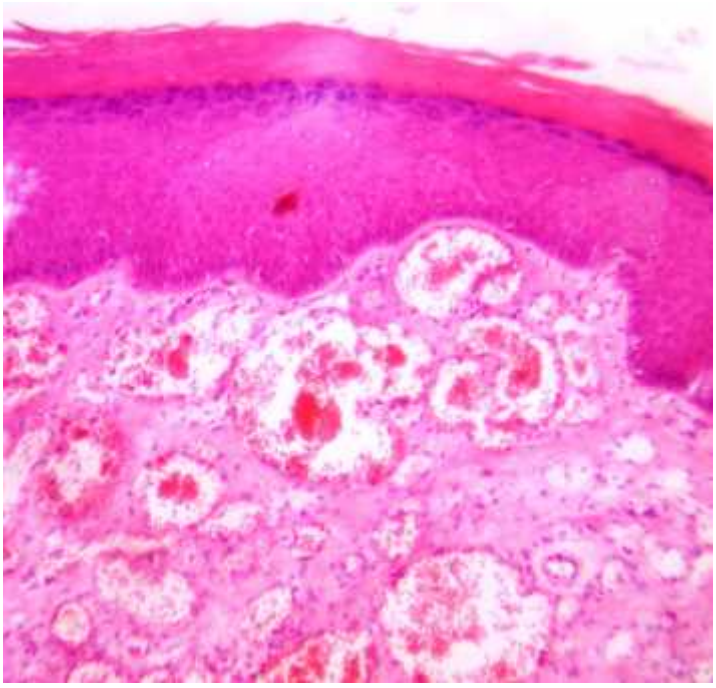


FIG 4: CAPILLARY HEMANGIOMA. H&E , (10x)

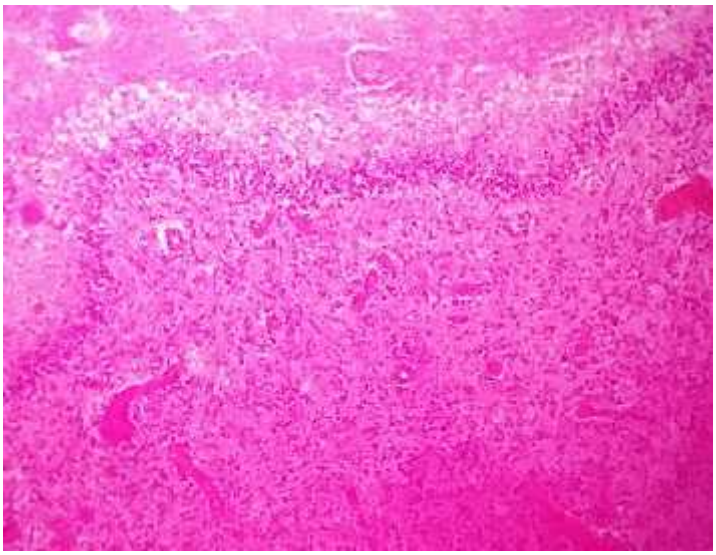


FIG 5: GLIOBLASTOMA MULTIFORME H&E , (10x)

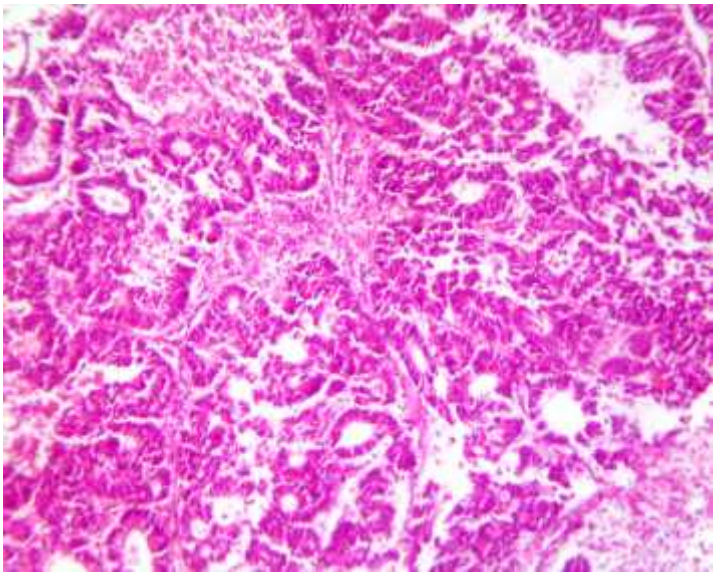


FIG 6:WELL DIFFERENTIATED ADENOCARCINOMA COLON H&E , (10x)

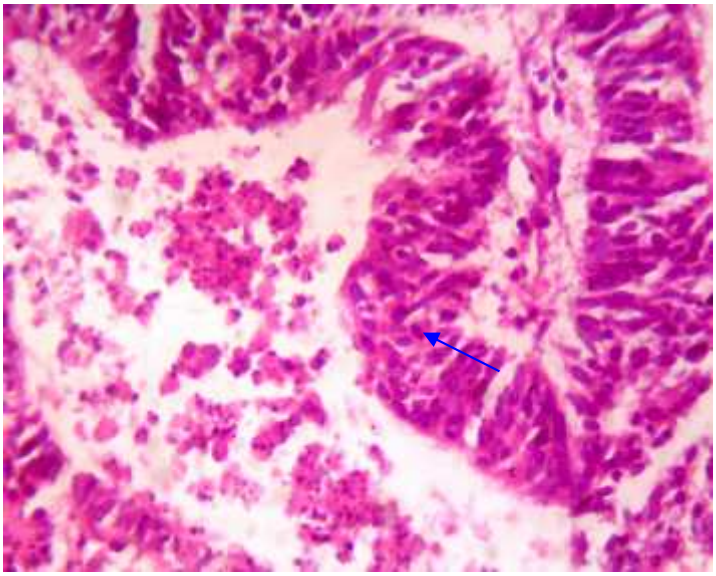


FIG 6a:WELL DIFFERENTIATED ADENOCARCINOMA COLON-LUMINAL MITOSIS H&E , (40x)

↗ **Luminal Mitosis**

FIG 7:ENDOMETRIOSIS H&E , (10x)

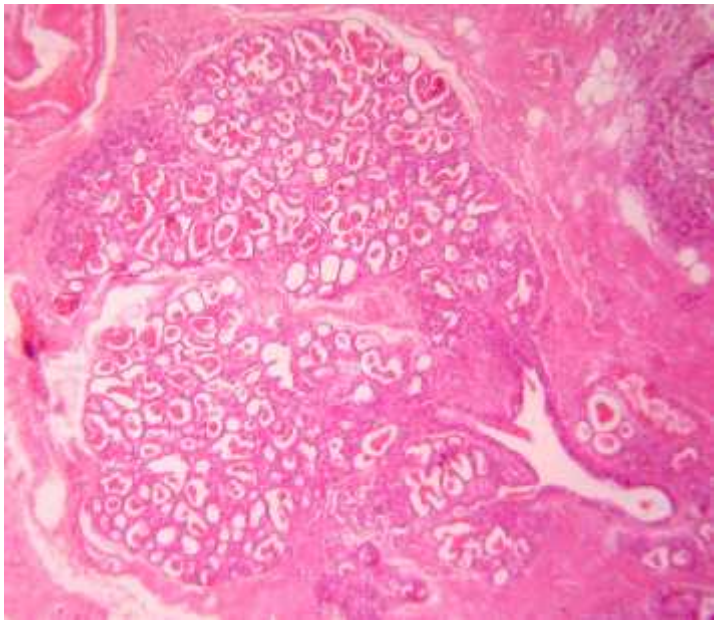


FIG 8:FIBROCYSTIC DISEASE WITH APOCRINE ADENOSIS,H&E , (10x)

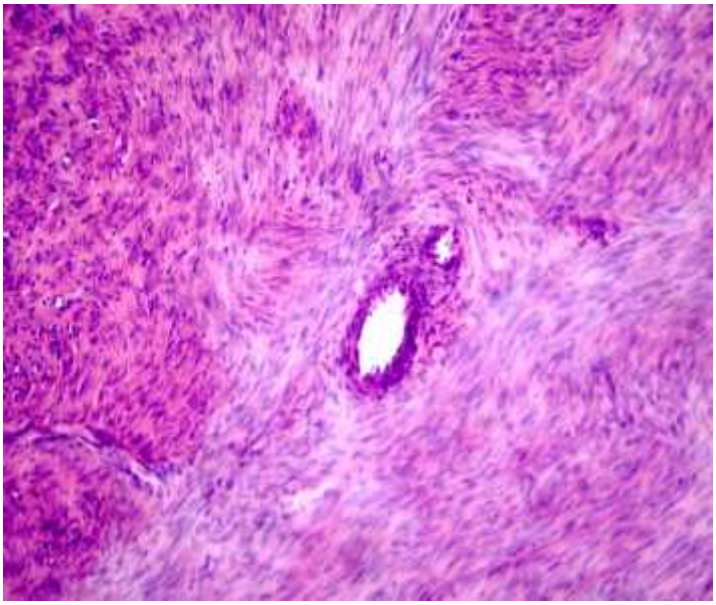


FIG 9:HYALINISED AREA OF LEIOMYOMA,H&E , (10x)

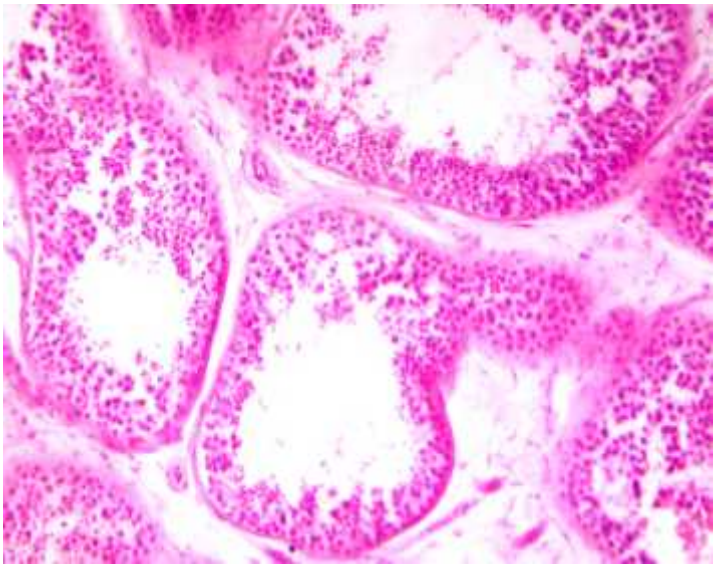


FIG 10: TESTIS H&E , (10x)

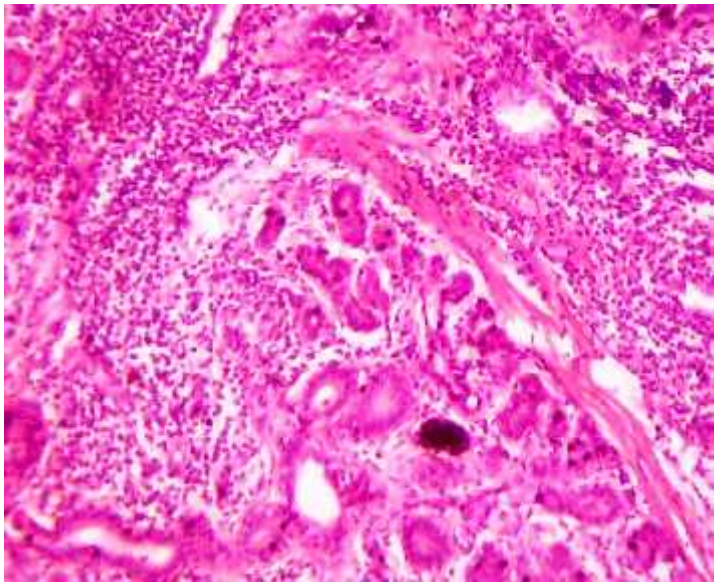


FIG 11: LYMPHOCYTIC SIALADENITIS H&E , (10x)

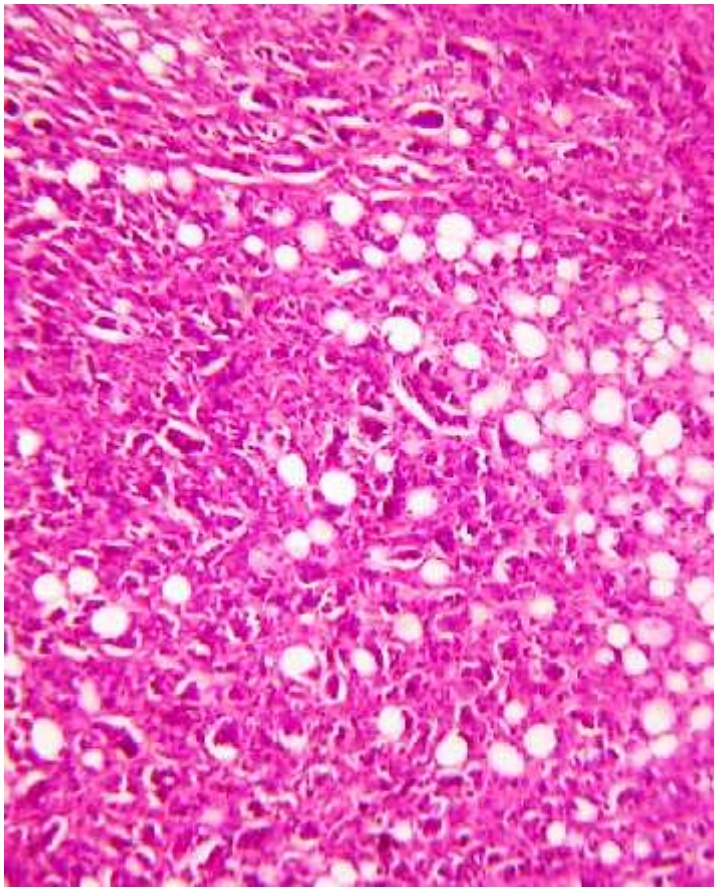


FIG 12: INVASIVE LOBULAR CARCINOMA BREAST H&E , (10x)

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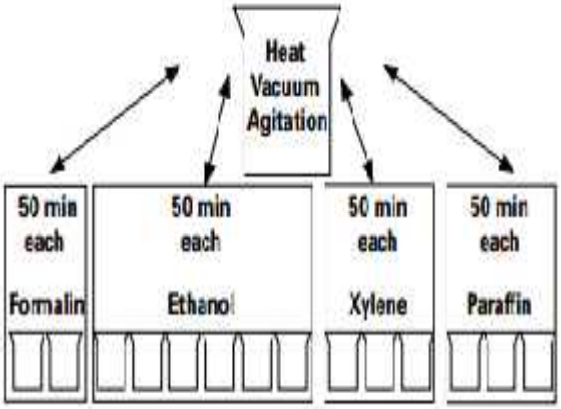

Figure 1 Schematic representation of the conventional overnight tissue processing method with a single retort and 14 stations that supply solutions to the retort. The exposure time is shown.

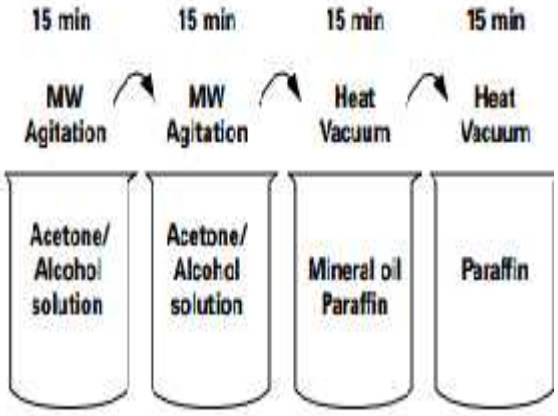

Figure 2 The rapid tissue processing method is relatively simple compared with the conventional overnight tissue processing method (Figure 1). MW, microwave.


BioWave Pro
BioWave Pro
PELCO

Sl. no	HPE NO	HOSP. NO	AGE	SEX	SPECIMEN	DIAGNOSIS	CATEGORY OF SPECIMEN	NEW COMPOUND FIXATIVE			FORMALIN		
								CYTOPLASM	NUCLEUS	ARCHITECTURE	CYTOPLASM	NUCLEUS	ARCHITECTURE
1	3099/16	57043	13	M	APPENDIX	ACUTE APPENDICITIS	INTERMEDIATE	3	3	3	3	3	3
2	3239/16	60092	30	F	THYROID	MULTI NODULAR GOITRE	INTERMEDIATE	3	3	3	3	3	3
3	3645/16	66985	41	F	ADIPOSE TISSUE	LIPOMA	SOFT	3	3	3	3	3	3
4	G2451/16	66639	60	F	CERVIX	CERVICAL INTRAEPITHELIAL NEOPLASIA II	HARD	3	3	3	3	3	3
5	G2841/16	78405	65	F	CERVIX	CHRONIC CERVICITIS	HARD	3	3	3	3	3	3
6	4444/16	78557	65	F	BREAST	ADENOMYOEPITHELIOMA	INTERMEDIATE	3	3	3	3	3	3
7	4481/16	79799	31	F	BREAST	IDC NOS	INTERMEDIATE	3	3	3	3	3	3
8	4481/16	79799	31	F	NODE	REACTIVE HYPERPLASIA	SOFT	3	3	2	3	3	3
9	4482/16	79214	65	F	KIDNEY	CHRONIC GRANULOMATOUS NEPHRITIS	INTERMEDIATE	3	3	3	3	3	3
10	FP3/17	25	55	M	BRAIN	NORMAL	INTERMEDIATE	3	3	3	3	3	3
11	FP4/17	26	60	M	BRAIN	NORMAL	INTERMEDIATE	3	3	3	3	3	3
12	314/17	1561	75	M	NASAL BIOPSY	INVERTED PAPILLOMA	SMALL BIOPSY	3	3	3	3	3	3
13	316/17	4733	20	F	NASAL POLYP	INFLAMMATORY POLYP	SMALL BIOPSY	3	3	3	3	3	3
14	321/17	1053	28	M	NODE	HODGKIN DISEASE	SOFT	3	3	2	3	3	3
15	535/17	5946	17	F	BONE	ORGANISING EXUDATE	DECALCIFIED	2	2	2	3	3	3
16	537/17	5816	13	M	BONE	OSTEOCHONDROMA	DECALCIFIED	2	2	2	3	3	3
17	538/17	9924	61	M	BONE	ANGIOMATOUS LESION	DECALCIFIED	2	2	2	3	3	3
18	556/17	5980	50	F	ADIPOSE TISSUE	NORMAL	SOFT	3	3	3	3	3	3
19	559/17	8908	52	F	THYROID	COLLOID RICH NODULAR GOITRE	INTERMEDIATE	3	3	3	3	3	3
20	561/17	8816	52	F	SIGMOID COLON	NORMAL	INTERMEDIATE	3	3	3	3	3	3
21	562/17	11002	29	M	SIGMOID COLON	HEMORRAGHIC NECROSIS	INTERMEDIATE	3	3	3	3	3	3
22	570/17	5953	41	M	PARIETAL SOL EXCISION	FIBROBLASTIC MENINGIOMA	SMALL BIOPSY	3	3	3	3	3	3

Sl. no	HPE NO	HOSP. NO	AGE	SEX	SPECIMEN	DIAGNOSIS	CATEGORY OF SPECIMEN	NEW COMPOUND FIXATIVE			FORMALIN		
								CYTOPLASM	NUCLEUS	ARCHITECTURE	CYTOPLASM	NUCLEUS	ARCHITECTURE
23	594/17	6267	53	F	GALL BLADDER	CHRONIC CALCULOUS CHOLECYSTITIS	INTERMEDIATE	3	3	3	3	3	3
24	597/17	52068	22	F	SKIN	EPIDERMAL INCLUSION CYST	HARD	3	3	3	3	3	3
25	598/17	6420	69	M	TURP	PROSTATIC ADENOCARCINOMA	SMALL BIOPSY	3	3	3	3	3	3
26	1307/17	22898	37	F	APPENDIX	ACUTE APPENDICITIS	INTERMEDIATE	3	3	3	3	3	3
27	1328/17	22963	34	F	THYROID	FOLLICULAR ADENOMA/COLLOID GOITRE	INTERMEDIATE	3	3	3	3	3	3
28	1329/17	22389	65	F	BREAST	INVASIVE LOBULAR CARCINOMA	INTERMEDIATE	3	3	3	3	3	3
29	1329/17	22389	65	F	NODE	METASTATIC CARCINOMATOUS DEPOSIT	SOFT	3	3	2	3	3	3
30	1336/17	23263	52	F	THYROID	PARATHYROID PARENCHYMA	INTERMEDIATE	3	3	3	3	3	3
31	1434/17	24008	42	M	TESTIS	AGE RELATED CHANGES	INTERMEDIATE	3	3	3	3	3	3
32	1457/17	20500	40	M	NODE	REACTIVE HYPERPLASIA	SOFT	3	3	2	3	3	3
33	1461/17	21278	50	M	LEG WIDE EXCISION	WELL DIFFERENTIATED SCC	INTERMEDIATE	3	3	3	3	3	3
34	1462/17	18644	57	F	TONGUE	NORMAL MUSCLE	HARD	3	3	3	3	3	3
35	FP88/17	620	6	MCH	AORTA	UNREMARKABLE	INTERMEDIATE	3	3	3	3	3	3
36	G1307/17	33328	47	F	CERVIX BIOPSY	INVASIVE SCC LARGE CELL NONKERATINISING TYPE	SMALL BIOPSY	3	3	3	3	3	3
37	1694/17	32899	64	M	PARATESTICULAR TISSUE	MALIGNANT MESOTHELIOMA	INTERMEDIATE	3	3	3	3	3	3
38	1697/17	26953	55	F	THYROID	FOLLICULAR ADENOMA/NODULAR GOITRE	INTERMEDIATE	3	3	3	3	3	3

Sl. no	HPE NO	HOSP. NO	AGE	SEX	SPECIMEN	DIAGNOSIS	CATEGORY OF SPECIMEN	NEW COMPOUND FIXATIVE			FORMALIN		
								CYTOPLASM	NUCLEUS	ARCHITECTURE	CYTOPLASM	NUCLEUS	ARCHITECTURE
39	1698/17	33302	35	F	BREAST	FIBROADENOMATOID HYPERPLASIA	INTERMEDIATE	3	3	3	3	3	3
40	G1382/17	26963	52	F	UTERUS	ATROPHIC ENDOMETRIUM	HARD	3	3	3	3	3	3
41	1704/17	32947	39	M	OROPHARYNX BIOPSY	WELL DIFFERENTIATED SCC	SMALL BIOPSY	3	3	3	3	3	3
42	1706/17	32905	46	F	BREAST	IDC NOS	INTERMEDIATE	3	3	3	3	3	3
43	1785/17	33641	16	M	SALIVARY GLAND	LYMPHOCYTIC SIALADENITIS	INTERMEDIATE	3	3	3	3	3	3
44	1794/17	33844	75	M	LOWER LIP BIOPSY	WELL DIFFERENTIATED SCC	SMALL BIOPSY	3	3	3	3	3	3
45	1818/17	33626	64	M	RIGHT HEMICOLON	ADENOCARCINOID	INTERMEDIATE	3	3	3	3	3	3
46	1846/17	29313	40	M	SALIVARY GLAND	CHRONIC SIALADENITIS	INTERMEDIATE	3	3	3	3	3	3
47	G1454/17	232345	32	F	CERVIX BIOPSY	CHRONIC NON SPECIFIC CERVICITIS	SMALL BIOPSY	3	3	3	3	3	3
48	FP87/17	601	47	M	LIVER	FATTY CHANGE	INTERMEDIATE	3	3	3	3	3	3
49	FP87/17	601	47	M	CORONARY ARTERY	GRADE I ATHEROSCLEROSIS	INTERMEDIATE	3	3	3	3	3	3
50	G1469/17	36607	28	F	PRODUCTS	CONSISTENT WITH PRODUCTS	SMALL BIOPSY	3	3	3	3	3	3
51	1855/17	36149	70	M	TURP	BENIGN ADENOMATOUS HYPERPLASIA	SMALL BIOPSY	3	3	3	3	3	3
52	G1476/17	33112	44	F	OVARY	SEROUS PAPILLARY CYSTADENOCARCINOMA	INTERMEDIATE	3	3	3	3	3	3
53	1861/17	30391	13	M	SKIN-THIGH	CAVERNOUS HEMANGIOMA	HARD	3	3	3	3	3	3
54	1865/17	31679	37	F	CERVIX	INVASIVE LARGE CELL NK SCC	HARD	3	3	3	3	3	3
55	G1479/17	34570	45	F	ENDOMETRIAL CURETTAGE	IRREGULARLY PROLIFERATIVE ENDOMETRIUM	SMALL BIOPSY	3	3	3	3	3	3
56	G1480/17	36518	23	F	FALLOPIAN TUBE	NORMAL	INTERMEDIATE	3	3	3	3	3	3

Sl. no	HPE NO	HOSP. NO	AGE	SEX	SPECIMEN	DIAGNOSIS	CATEGORY OF SPECIMEN	NEW COMPOUND FIXATIVE			FORMALIN		
								CYTOPLASM	NUCLEUS	ARCHITECTURE	CYTOPLASM	NUCLEUS	ARCHITECTURE
57	1875/17	38624	45	M	HARD PALATE BIOPSY	ULCER WITH INFLAMMATORY GRANULATION TISSUE	SMALL BIOPSY	3	3	3	3	3	3
58	G1485/17	33199	45	F	OVARY	ENDOSALPINGIOSIS	INTERMEDIATE	3	3	3	3	3	3
59	1890/17	36556	22	M	SALIVARY GLAND	LYMPHOCYTIC SIALADENITIS	INTERMEDIATE	3	3	3	3	3	3
60	G1487/17	33841	48	F	ENDOMETRIAL CURETTAGE	IRREGULARLY PROLIFERATIVE ENDOMETRIUM	SMALL BIOPSY	3	3	3	3	3	3
61	1906/17	34951	44	F	GALL BLADDER	CHRONIC CHOLECYSTITIS	INTERMEDIATE	3	3	3	3	3	3
62	1914/17	33870	65	M	PENIS	INVASIVE SCC	INTERMEDIATE	3	3	3	3	3	3
63	2020/17	38832	80	M	TESTIS	AGE RELATED CHANGES	INTERMEDIATE	3	3	3	3	3	3
64	G1546/17	38165	48	F	PRODUCTS	PARTIAL MOLE	SMALL BIOPSY	3	3	3	3	3	3
65	G1547/17	37511	45	F	UTERUS	PROLIFERATIVE ENDOMETRIUM WITH INTRAMURAL LEIOMYOMA	HARD	3	3	3	3	3	3
66	G1555/17	37557	30	F	FALLOPIAN TUBE	NORMAL	INTERMEDIATE	3	3	3	3	3	3
67	2031/17	39213	64	F	GALL BLADDER	CHRONIC CALCULOUS CHOLECYSTITIS	INTERMEDIATE	3	3	3	3	3	3
68	2071/17	38774	54	M	EPIDIDYMIS	SIMPLE EPIDIDYMAL CYST	INTERMEDIATE	3	3	3	3	3	3
69	2168/17	40445	55	M	PROSTATE	BENIGN ADENOMYOMATOUS HYPERPLASIA	INTERMEDIATE	3	3	3	3	3	3
70	G1701/17	39687	48	F	ENDOMETRIAL CURETTAGE	EARLY SECRETORY ENDOMETRIUM	SMALL BIOPSY	3	3	3	3	3	3
71	FP98/17	686	36	M	AORTA	FATTY STREAK	INTERMEDIATE	3	3	3	3	3	3
72	FP98/17	686	36	M	LUNG	CONGESTION	INTERMEDIATE	3	3	3	3	3	3
73	FP98/17	686	36	M	KIDNEY	NORMAL	INTERMEDIATE	3	3	3	3	3	3

Sl. no	HPE NO	HOSP. NO	AGE	SEX	SPECIMEN	DIAGNOSIS	CATEGORY OF SPECIMEN	NEW COMPOUND FIXATIVE			FORMALIN		
								CYTOPLASM	NUCLEUS	ARCHITECTURE	CYTOPLASM	NUCLEUS	ARCHITECTURE
74	FP98/17	686	36	M	BRAIN	NORMAL	INTERMEDIATE	3	3	3	3	3	3
75	FP98/17	686	36	M	SPLEEN	CONGESTION	INTERMEDIATE	3	3	3	3	3	3
76	2287/17	41597	25	F	BREAST	FIBROADENOMATOID HYPERPLASIA	INTERMEDIATE	3	3	3	3	3	3
77	2305/17	41830	52	F	THYROID	NODULAR GOITRE	INTERMEDIATE	3	3	3	3	3	3
78	2310/17	40155	53	F	MUSCLE	NORMAL	INTERMEDIATE	3	3	3	3	3	3
79	2323/17	33870	65	M	TESTIS	AGE RELATED CHANGES	INTERMEDIATE	3	3	3	3	3	3
80	2325/17	41331	52	F	UTERUS	WELL DIFFERENTIATED	HARD	3	3	3	3	3	3
81	2325/17	41331	52	F	OVARY	FOLLICULAR CYST	INTERMEDIATE	3	3	3	3	3	3
82	2356/17	44890	45	F	TONSIL	REACTIVE FOLLICULAR HYPERPLASIA	INTERMEDIATE	3	3	3	3	3	3
83	2357/17	45409	37	F	TONSIL	REACTIVE FOLLICULAR HYPERPLASIA	INTERMEDIATE	3	3	3	3	3	3
84	G2114/17	51764	40	F	OVARY	FOLLICULAR CYST	INTERMEDIATE	3	3	3	3	3	3
85	G2126/17	53985	20	F	CERVIX	NORMAL HISTOMORPHOLOGY	HARD	3	3	3	3	3	3
86	G2148/17	50133	45	F	UTERUS	LEIOMYOMA	HARD	3	3	3	3	3	3
87	G2148/17	50133	45	F	OVARY	CORPUS LUTEAL CYST	INTERMEDIATE	3	3	3	3	3	3
88	2898/17	55019	37	M	SKIN	PSEUDOEPITHELIOMATOUS HYPERPLASIA	HARD	3	3	3	3	3	3
89	2899/17	172626	50	F	SKIN	NORMAL	HARD	3	3	3	3	3	3
90	2901/17	49225	67	M	TURBT	TRANSITIONAL CELL CARCINOMA	SMALL BIOPSY	3	3	3	3	3	3
91	2985/17	5472	mor	MCH	KIDNEY	WILMS TUMOR	INTERMEDIATE	3	3	3	3	3	3

Sl. no	HPE NO	HOSP. NO	AGE	SEX	SPECIMEN	DIAGNOSIS	CATEGORY OF SPECIMEN	NEW COMPOUND FIXATIVE			FORMALIN		
								CYTOPLASM	NUCLEUS	ARCHITECTURE	CYTOPLASM	NUCLEUS	ARCHITECTURE
92	2986/17	177751	77	M	NASAL SOFT TISSUE	MUCORMYCOSIS	SMALL BIOPSY	3	3	3	3	3	3
93	2994/17	54259	34	F	PARIETAL WALL TUMOR	ENDOMETRIOSIS	SMALL BIOPSY	3	3	3	3	3	3
94	G2246/17	53899	38	F	UTERUS	PSEUDODECIDUALISATION OF ENDOMETRIUM	HARD	3	3	3	3	3	3
95	3011/17	56276	30	F	BREAST	FIBROCYSTIC DISEASE WITH APOCRINE ADENOSIS AND GRANULOMATOUS MASTITIS	INTERMEDIATE	3	3	3	3	3	3
96	3012/17	52644	65	F	SKIN	BASAL CELL CARCINOMA	HARD	3	3	3	3	3	3
97	3027/17	55428	80	F	NASAL POLYP	ALLERGIC POLYP	INTERMEDIATE	3	3	3	3	3	3
98	3076/17	57426	51	F	COLON	WELL DIFFERENTIATED ADENOCARCINOMA	INTERMEDIATE	3	3	3	3	3	3
99	3092/17	56724	27	M	SOL	GLIOBLASTOMA MULTIFORME	SMALL BIOPSY	3	3	3	3	3	3
100	3098/17	70710	69	M	NAIL	NORMAL	HARD	3	3	3	3	3	3